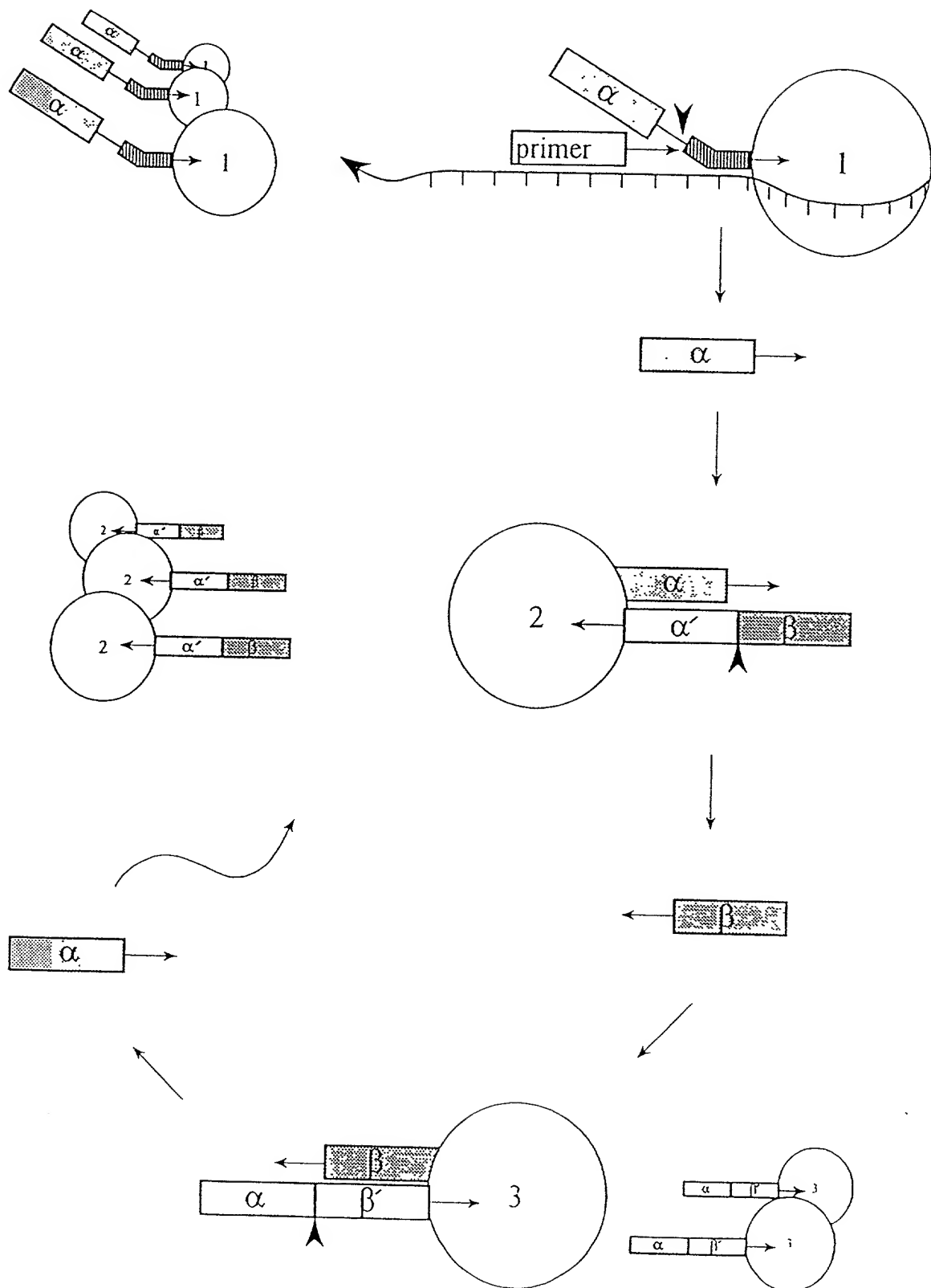


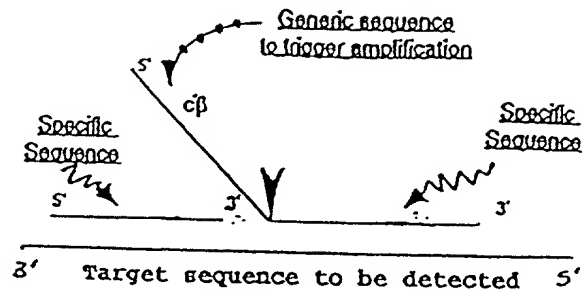
FIGURE 1A



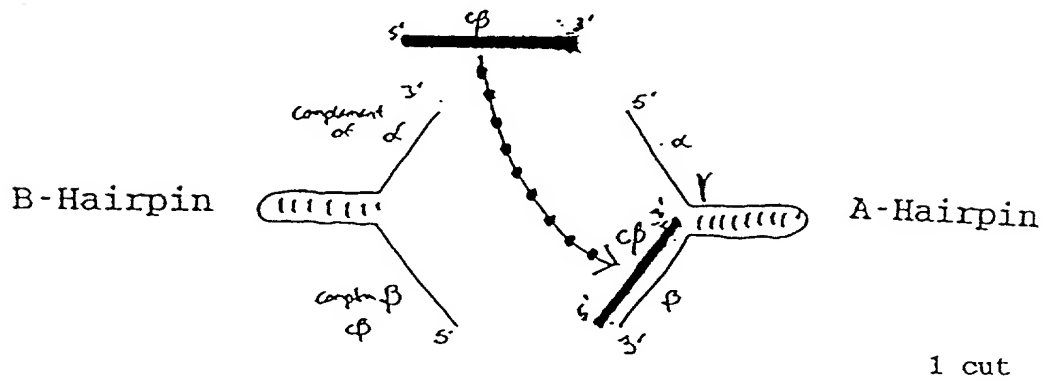
10074323 021203 202120 32E4C001

FIGURE 1B

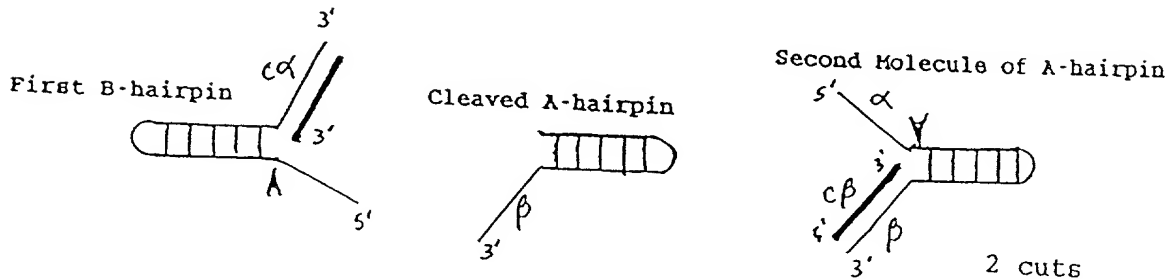
PART ONE: TRIGGER REACTION



PART TWO: DETECTION REACTION



Denature, anneal



Denature, anneal

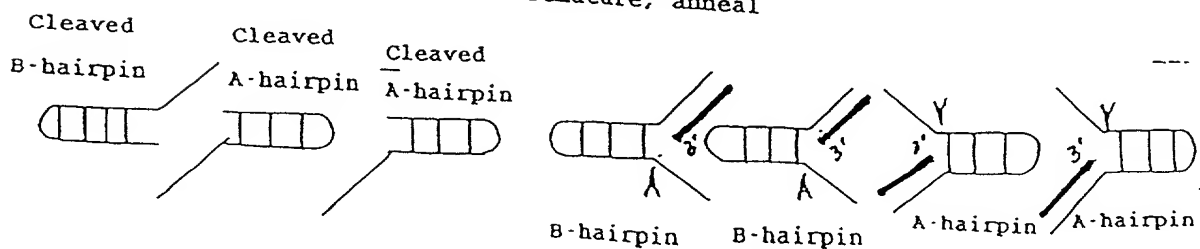


FIGURE 2 (cont'd)

MAJORITY (SEQ ID NO:7)	TCCAGGCCCCACATCGGAXGACCTGAXGCTCTCCTGGGAGGCTXTCGCAGGTGGCCACCGACCTCGCCCTGGA	
DNAPTAA (SEQ ID NO:1)	...T.....C..T...A.....C..GG..A.....	764
DNAPTTL (SEQ ID NO:2)	...GGG.....G.C...GCC..T..C..A...T.....A...T.....	761
DNAPTHH (SEQ ID NO:3)	..A.....C.....A.....C.G.....T.....C.....G.....C.....	770
MAJORITY	CGTGGACCTTCGCCCAAGXGGGGGAGCCCGACCGGGAGGGGCTTAGGGCCCTTCTGGAGAGGCTGGAGTTT	
DNAPTAA (SEQ ID NO:1)	...AA.....A.....A.....A.....T.....T.....	834
DNAPTTL (SEQ ID NO:2)	...GG.G.C.C..CACA...A...T.....T..GC...T...T...C..T.....	831
DNAPTHH (SEQ ID NO:3)	...C.....C..G.....G.....G.....C.....C.....	840
MAJORITY	GGCAGCCCTCGTCCAGGAGTTGGGGCTCGTGGAGGGGGCCCAAGGCCCTGGAGGAGGCCCGCCCTGGCCCGCCG	
DNAPTAA (SEQ ID NO:1)	...T.....T.....AA.....	904
DNAPTTL (SEQ ID NO:2)	..A.....G.....G..G...GCCA.....T..	901
DNAPTHH (SEQ ID NO:3)	...G.....C.....GCCG.....	910
MAJORITY	CGGAAGGGGCCCTTCGTGGCCCTTCGTCCCTTCGCCCGCCCGAGCCCATGTGGCCCGGAGGCTTCTGGCCCTGGC	
DNAPTAA (SEQ ID NO:1)	...T.....G.....AAG.....T.....	974
DNAPTTL (SEQ ID NO:2)	...TT.....TC.T.....T.....	971
DNAPTHH (SEQ ID NO:3)	...C.....C.....G.....AAA.....	980
MAJORITY	CGCCGCCAGGGGAGGGGGCGGCTCCACCGGGGCGACGACGCCCTTAXGGGCCCTXAGCGACCTXAAGGAGGCTG	
DNAPTAA (SEQ ID NO:1)	...G.....G.....C..C..G..T.A..AA.C...C.....G.....C..	1044
DNAPTTL (SEQ ID NO:2)	T.GG...GT.....G..CC...T.....A.....C.....G.....T.....G.....	1041
DNAPTHH (SEQ ID NO:3)	...TG.....C.....G.....G.....GCG...G..A.A.....C.....C.....	1050

5

FIGURE 2 (cont'd)

MAJORITY	(SEQ ID NO:7)	CGGGGCGTCCCTCGCCCAAGGACCTGGCCGTTTGGCCCTGAGCGAGGGCGCTXGACCTCTGCCCCGGGACG	
DNAPTAQ	(SEQ ID NO:1)G..T.....A.....AG.....C.....A.....T.G.....CG.....C.....	1111
DNAPTR	(SEQ ID NO:2)AA.....G.....G.....C.....G.....T.C..A.A.....	1120
DNAPTH	(SEQ ID NO:3)C.....C.....C.....TC.....G..A.....G.....	
MAJORITY		ACCCCATGCTCCTCGCCCTACCTCCTCGACCCCTCCAAACACCCACCCCGAGGGGTGGCCCCGGCGCTACGG	
DNAPTAQ	T.....	1184
DNAPTR	G.....T.....T.....T.....	1181
DNAPTH	G.....G.....G.....	1190
MAJORITY		GGGGGAGTGGACGGAGGAXGGCGGGGAGCGGGCCCTCCTXTCCGAGAGGCTCTTCGXGAACCTXXGGGAG	
DNAPTAQ		G.....G.....G.....GC.....T.....GGC.....GTG..G..	1254
DNAPTR	T.....A.....GG.....C.G.....A..C...AAA....	1251
DNAPTH	C..C.CCC.C.....C..G.....CAT.G.....CCTTA..	1260
MAJORITY		CGCCTTGAGGGGAGGAGAGGCTCCTTTGGCTTTACGAGGAGCTCGAGAGCCCTTTCCCGGCTCCTCG	
DNAPTAQ		A.G.....G.....G.....G.....GCT.....	1324
DNAPTR	A..A..A..AC.G..G.....G.....G.....GT...	1321
DNAPTH	C.....A.....C.....G.....A.....G.....	1330
MAJORITY		CCCACATCGAGGCCACGGGGGTXCGGGCTGGACGTGGCCCTACCTCGAGGGCGCTXTCCTTGGAGGCTGGCGGA	
DNAPTAQ	G..C.....T...AG...T.G.....C..	1394
DNAPTR	GG.....C.....C.....C.....A..C	1391
DNAPTH	C.....A.....T.....T.....C.T.....	1400

FIGURE 2 (cont'd)

MAJORITY (SEQ ID NO:7)	GGAGATCGCGCGCGCTCGAGGAGGAGGTCTTCCGGCTGGCGCGGCGCACCCCTTCAACCTCAACTCCCGGGGAC	
DNAPTAO (SEQ ID NO:1)GC.....GC.....	1464
DNAPTFL (SEQ ID NO:2)G.G.....AG..G.....	1461
DNAPTTH (SEQ ID NO:3)T.....T.....G.....	1472
MAJORITY	CAGCTGGAAAGCGTGCCTTTGACGAGCTXGGGGCTTCCGGCCATCGGCAAGAGCGGAGAGACXGGCAAGC	
DNAPTAOC.....C.....A.....	1534
DNAPTFLGC.....G.C..G..T.....	1531
DNAPTTHTA.....T.G..G.....C.A.....	1540
MAJORITY	GCCTCCACCGCGCGCGCTGCTGGAGGCGCTXCGXGAGGCGCGCACCCCATCGTGGAGAGAGATCCTGCAGTA	
DNAPTAOC.....C.....C.....	1604
DNAPTFLT.....G..A.....GCGC.....	1601
DNAPTTHG.....A..G.....C.....C..C..	1610
MAJORITY	CGCGGAGCTCACCAAGCTCAAGAACACCTACATXGACCGCGCTGCCXGXCTCGTCCACCCCGAGGACGGCG	
DNAPTAOG.....G.....T.....G.A.....A.....	1617
DNAPTFLA.....A.....G.C.....A.....C.....	1616
DNAPTTHG.C.....C.AAG.....G.....	1680
MAJORITY	CGCCCTCCACACCGCGCTTCAACCCAGACGGCGCGCACCGGCGAGGCTTAGTAGCTCCGACCCCAACCTGC	
DNAPTAOA.....A.....T.....C..	1744
DNAPTFLG.....C.....TCG.....	1741
DNAPTTHG.....G.....	1750

FIGURE 2 (cont'd)

MAJORITY (SEQ ID NO:7)	AGAACATCCCGCTCCGCCACCCXCTGGCCCAAGGATCCGCCCGGCCCTTCGTGCCCGAGGACGGCTGGCT	
DNAPTAA (SEQ ID NO:1)G..T..G.....A..C.....C...C..	1814
DNAPTTL (SEQ ID NO:2)G.....T.....C..C.....A.....C.....C.....	1811
DNAPTTH (SEQ ID NO:3)CT.....C.....C.....C.....C.....C.....	1812
MAJORITY	GTGGTGGCGCTGGACTATAGCCAGATAGAGCTCCGGTCCGTGGCCGACCTCTCCGGGGAGGAGAACCTG	
DNAPTAA	A.....T..T.....G.....A.....G.....C.....C.....	1884
DNAPTTL	.C.....T..T.....G.....T.....T.....C.....C.....	1881
DNAPTTHC.....C.....C.....C.....C.....A.....C.....	1890
MAJORITY	ATCCCGGCTCTCCAGAGGGAGGACATCCACACCCAGACCCGACCTGGATGTTCCGGCTCCCGCCCGG	
DNAPTAAC.....C.....GG.....C.....C.....G...G...	1954
DNAPTTLT.....T.....C.....C.....C.....T.....T.....C...	1951
DNAPTTH	A.....A.....A.....A.....A.....A.....C.....C.....	1960
MAJORITY	AGCGCGTGGACCCCTGATCGCGCGGGGGCCAGACCATCAACTTCGGGCTCCTGTACGGCATGTCCGG	
DNAPTAAA..GG..A.....T.....C.....C.....C.....C.....G...	2021
DNAPTTLA..GG..A.....T.....C.....C.....C.....C.....G...	2021
DNAPTTHA..GG..A.....T.....C.....C.....C.....C.....G...	2030
MAJORITY	CCACCGGCTCTCCAGGAGCTTCCCATCCCGTACCGAGGAGGGCGGTGGCGCTTCATTGAGCGGCTACTTCCAG	
DNAPTAAA.....A.....T.....C.....C.....C.....C.....T...	2094
DNAPTTLGG.....T.....C.....C.....C.....C.....C.....	2091
DNAPTTH	...TA..G.....C.....C.....C.....C.....C.....T...A.....A.....A.....	2100

8

FIGURE 2 (cont'd)

MAJORITY (SEQ ID NO:7)	AGCTTCCCCAAAGGTGGGGCCCTGGATTGAGAAAGACCCCTGGAGGAGGGGAGGGGGGCTACCTGGAGA	
DNAPTAO (SEQ ID NO:1)	2164
DNAPTFL (SEQ ID NO:2)	2161
DNAPTTH (SEQ ID NO:3)	2177
MAJORITY	CCCTCTTGGGGCCGGGGCTAGGTGGCCGACCTCAACCCCGGGTGAAGAGCGTGGGGGAGGGGGCGGA	
DNAPTAO	2234
DNAPTFL	2231
DNAPTTH	2240
MAJORITY	GGCGATGGCCCTTCAACATGCCCGTCCAGGGCACCGCCCGGACCTCATGAAGCTGGCCCATGGTGAAGCTC	
DNAPTAO	2304
DNAPTFL	2301
DNAPTTH	2310
MAJORITY	TTCCCGCCGGCTXCAGGAAATGGGGGCCAGGATGCTCCTXCAGGTCCACGACGAGCTGCTCCTCGAGGGCC	
DNAPTAO	2371
DNAPTFL	2371
DNAPTTH	2380
MAJORITY	CCAAAGAGCGGGCGGAGGCGGTGGCCCGCTTGGCCCAAGGAGGTGATGGAGGGGGTCTATCCCGCTGGCGGT	
DNAPTAO	2444
DNAPTFL	2441
DNAPTTH	2450

FIGURE 2 (cont'd)

MAJORITY (SEQ ID NO:7)	GGCCCTGGAGGTGGAGGTGGGGATGGGGGAGGACTGGCTCTCCGCCAAGGAGTAG	
DNAPTAA (SEQ ID NO:1)A.....	GA
DNAPTLL (SEQ ID NO:2)CC.....	GT...
DNAPTTH (SEQ ID NO:3)T.....	GT...

2499
2496
2505

FIGURE 3 (cont'd)

MAJORITY (SEQ ID NO:8)	RGLLAKOLAVLALREGLDLXPGDDPMLLAYLLDPSNTTPEGVARRYGGEWTEADAGERALLSERLFXNLXX	
TAQ PRO (SEQ ID NO:4)	S.....G.P.....E.....A.....A.....WG	44
TRL PRO (SEQ ID NO:5)	I.....F.E.....A.....QT.KE	45
TTH PRO (SEQ ID NO:6)	S.....V.....AH.....HR..LK	420
MAJORITY	RLEGEERLLWLYXEVEKPLSRVLAHMEATGVRLDVAYLOALSLEVAEEI RRLEEEVFRLAGHPFNLNSRD	
TAQ PRO	R...R...A.....R.....A.....A.....	488
TRL PRO	K.....E.....R.....EA.V.O.....	487
TTH PRO	K.....H.....L.....	490
MAJORITY	QLERVLFDELGLPAIGKTEKTGKRSTSAAVLEALREAHPIVEKILQYRELTCLKNTYIDPLPXLVHPRTG	
TAQ PROS.....S.....D.I.....	558
TRL PRODR.....A.....K..	557
TTH PRO	R...L...Q.....H.....V.....S.....	560
MAJORITY	RLHTRFNOTATATGRLSSSDPNLONI PVRTPLGQRI RRAFFVAEEGWXLVALDYSOIELRVLAHLSCDENL	
TAQ PROL.....I.....L.....	628
TRL PROV.....V.....	627
TTH PROA.....A.....	630
MAJORITY	IRVFQEGRDIHTQTASWNFGVPPPEAVDPLMRRAAKTI NFGVLGYGMSAHRLSOELAI PYEEAVAFIERYFO	
TAQ PRO	E.....R.....Q.....	698
TRL PRO	S...G.....G...S.....	697
TTH PRO	K.....V.....	700

FIGURE 3 (cont'd)

MAJORITY (SEQ ID NO:8)	SFPKVRAWIEKTL E E G R R R G Y V E T L F G R R R Y V P D L N A R V K S V R E A A E R M A F N M P V O G T A A D L M K L A M V K L	
TAQ PRQ (SEQ ID NO:4) E.....	70
TRL PRQ (SEQ ID NO:5)	. Y..... G.....	767
TTH PRQ (SEQ ID NO:6) K.....	770
MAJORITY	F P R L X E M G A R M L L Q V H D E L V L E A P K X R A E X V A A L A K E V M E G V Y P L A V P L E V E V G X G E D W L S A K E X	
TAQ PRQ E..... A..... R.....	833
TRL PRQ Q..... L..... D..... R..... W..... Q.....	831
TTH PRQ R..... L..... Q A..... E..... A..... K A..... M..... G.....	835

Genes for Wild-Type and Pol(-) DNAPTaq

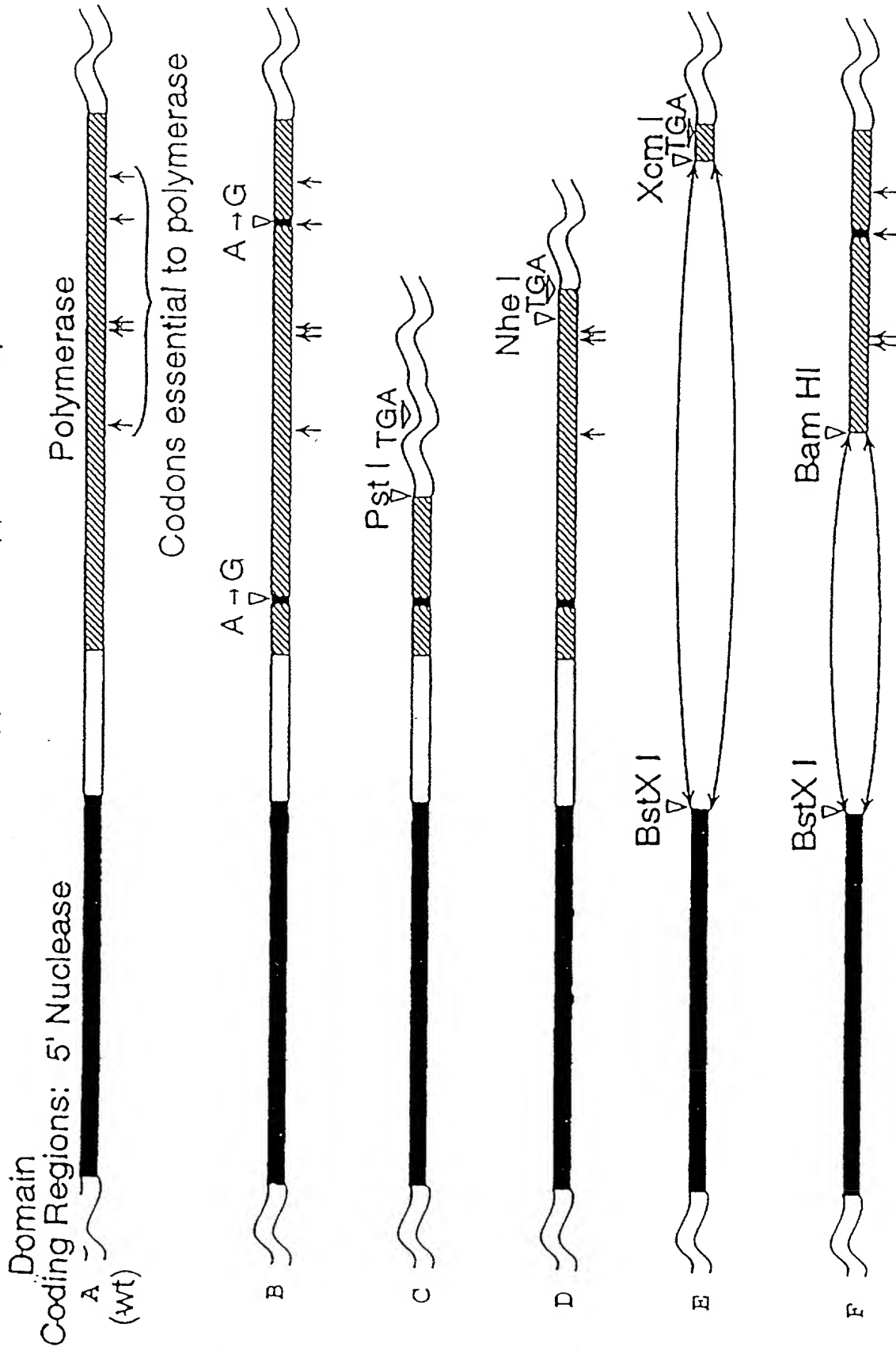


FIGURE 5

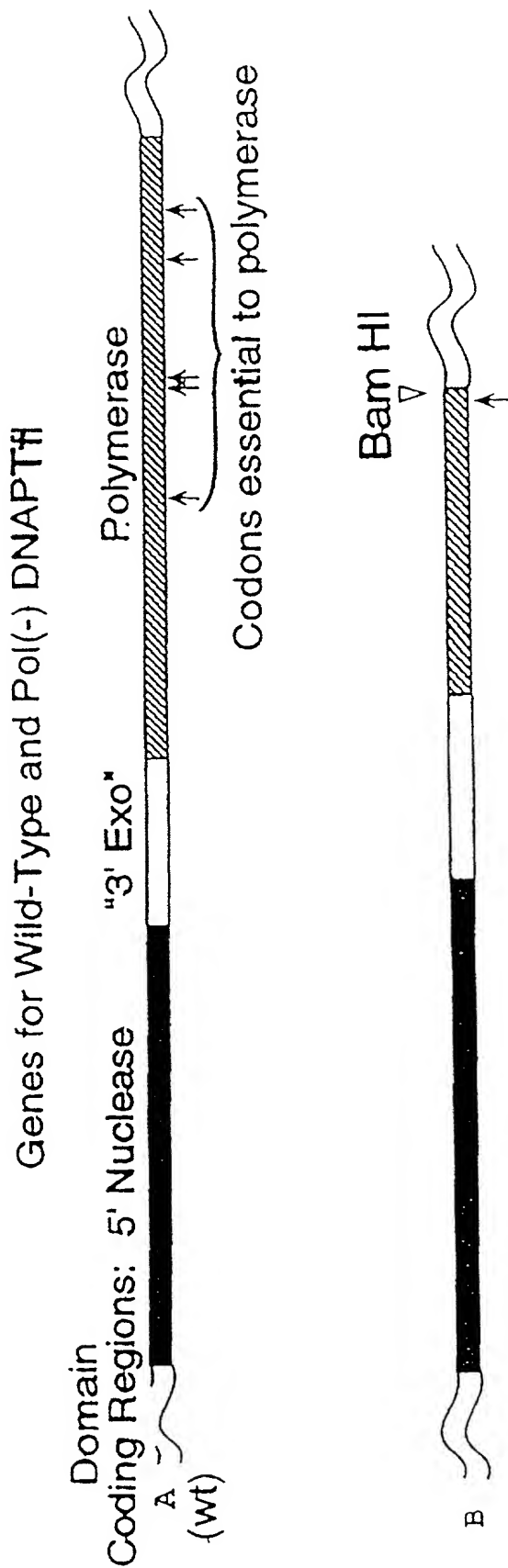


FIGURE 6

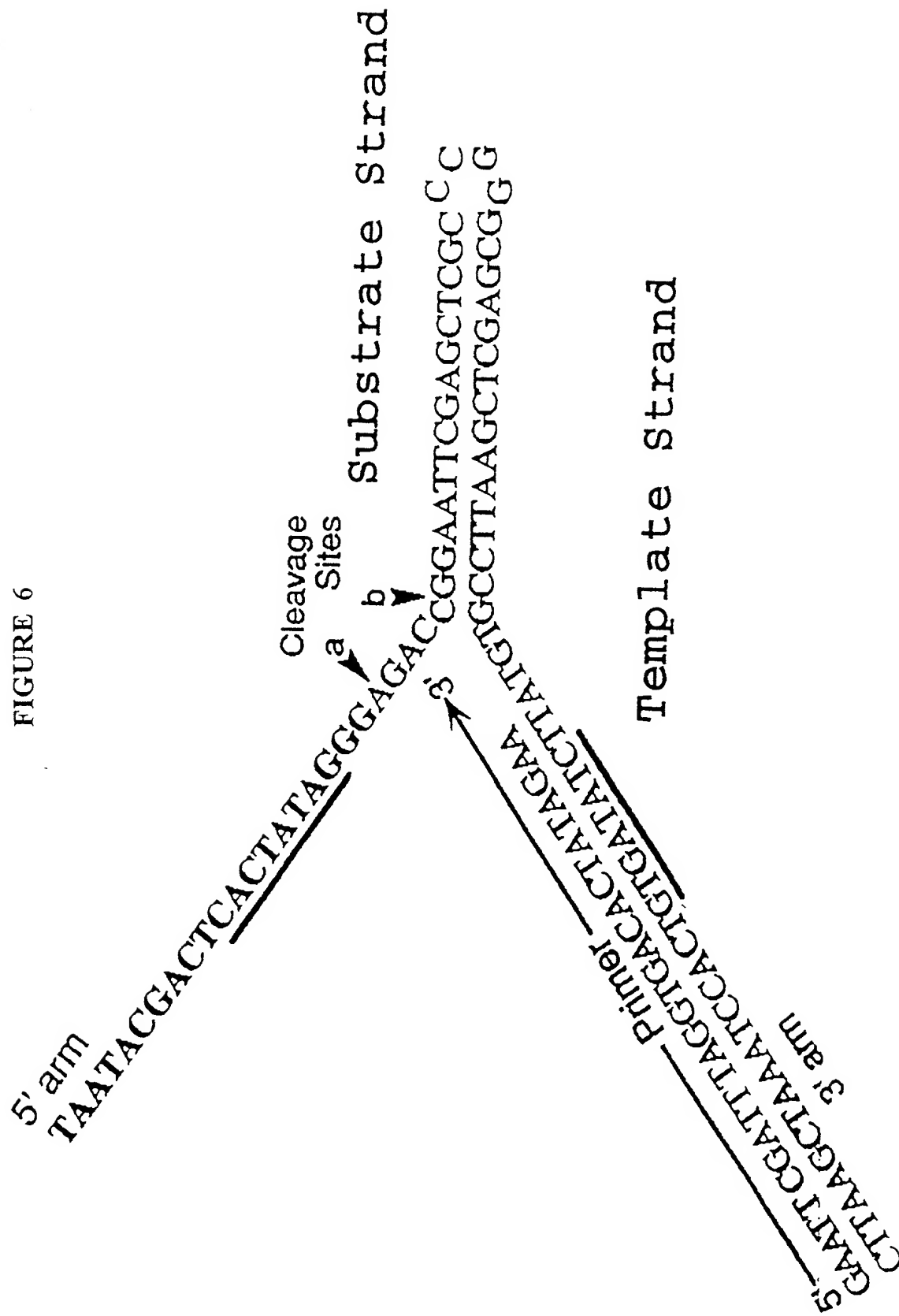


FIGURE 7



228	229	230	231	232	233	234	235	236	237	238	239	240	241	242	243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	276	277	278	279	280	281	282	283	284	285	286	287	288	289	290	291	292	293	294	295	296	297	298	299	300	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	316	317	318	319	320	321	322	323	324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350	351	352	353	354	355	356	357	358	359	360	361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376	377	378	379	380	381	382	383	384	385	386	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	413	414	415	416	417	418	419	420	421	422	423	424	425	426	427	428	429	430	431	432	433	434	435	436	437	438	439	440	441	442	443	444	445	446	447	448	449	450	451	452	453	454	455	456	457	458	459	460	461	462	463	464	465	466	467	468	469	470	471	472	473	474	475	476	477	478	479	480	481	482	483	484	485	486	487	488	489	490	491	492	493	494	495	496	497	498	499	500	501	502	503	504	505	506	507	508	509	510	511	512	513	514	515	516	517	518	519	520	521	522	523	524	525	526	527	528	529	530	531	532	533	534	535	536	537	538	539	540	541	542	543	544	545	546	547	548	549	550	551	552	553	554	555	556	557	558	559	560	561	562	563	564	565	566	567	568	569	570	571	572	573	574	575	576	577	578	579	580	581	582	583	584	585	586	587	588	589	590	591	592	593	594	595	596	597	598	599	600	601	602	603	604	605	606	607	608	609	610	611	612	613	614	615	616	617	618	619	620	621	622	623	624	625	626	627	628	629	630	631	632	633	634	635	636	637	638	639	640	641	642	643	644	645	646	647	648	649	650	651	652	653	654	655	656	657	658	659	660	661	662	663	664	665	666	667	668	669	670	671	672	673	674	675	676	677	678	679	680	681
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FIGURE 9

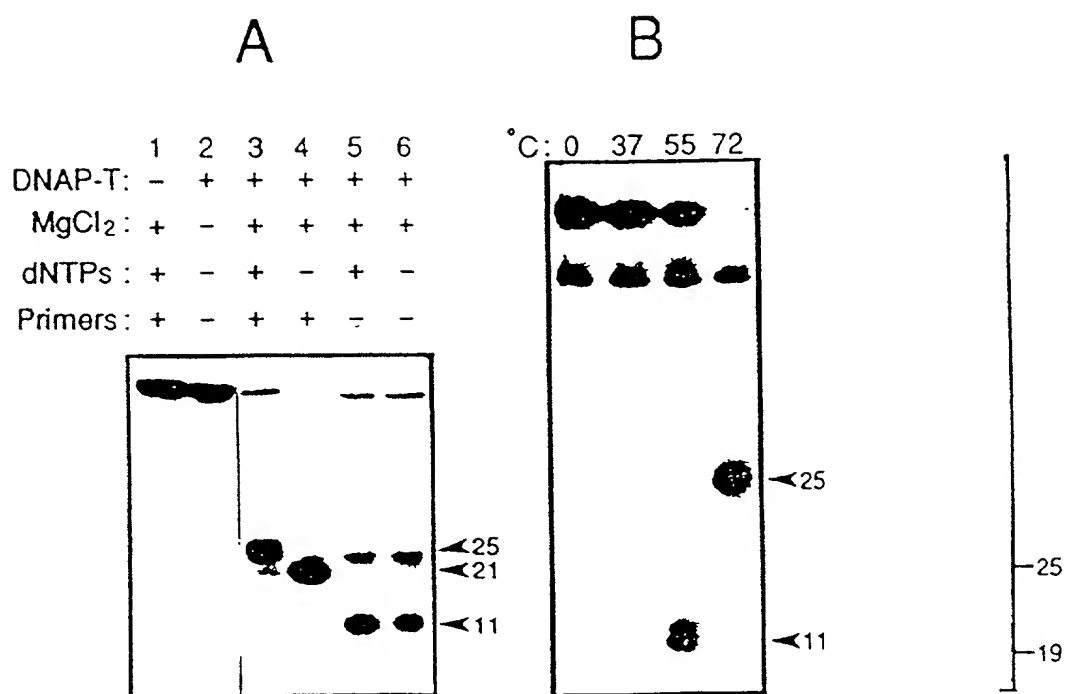


FIGURE 10

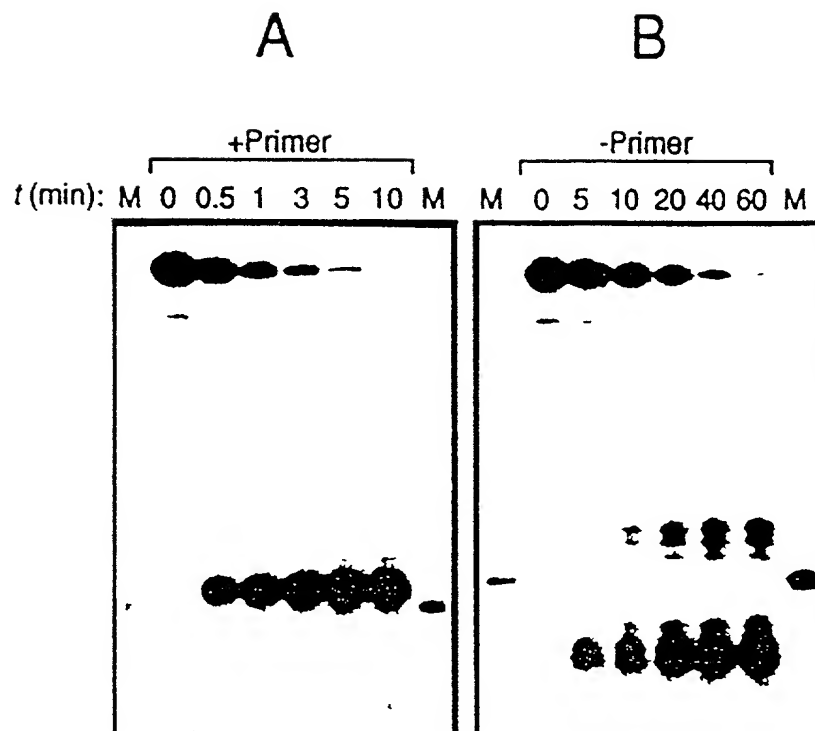


FIGURE 11

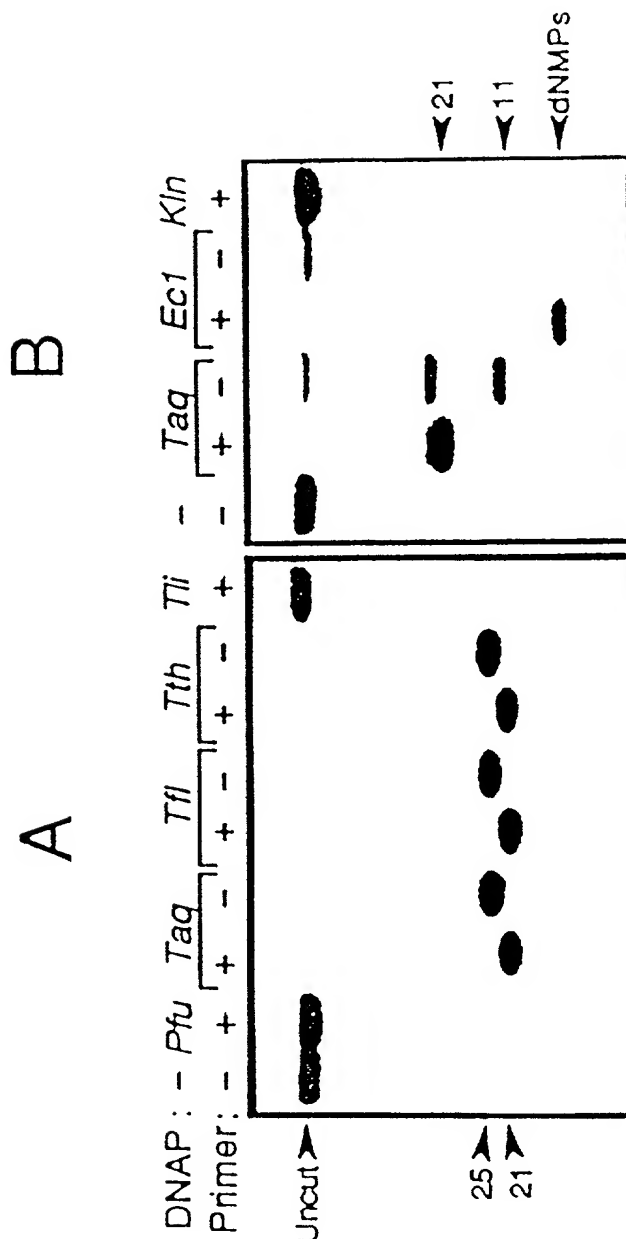


FIGURE 12

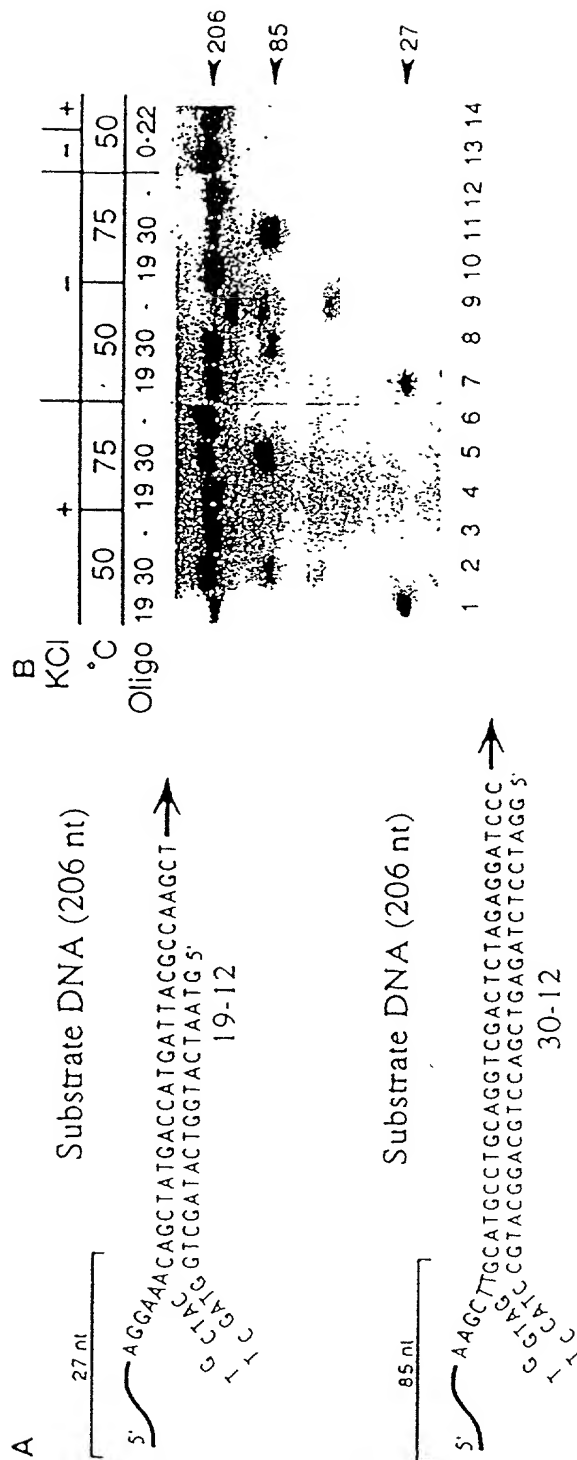


FIGURE 13

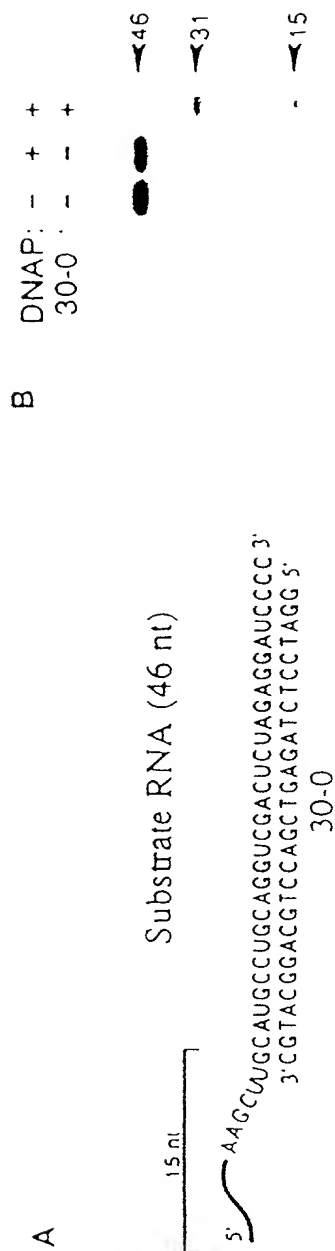


FIGURE 14

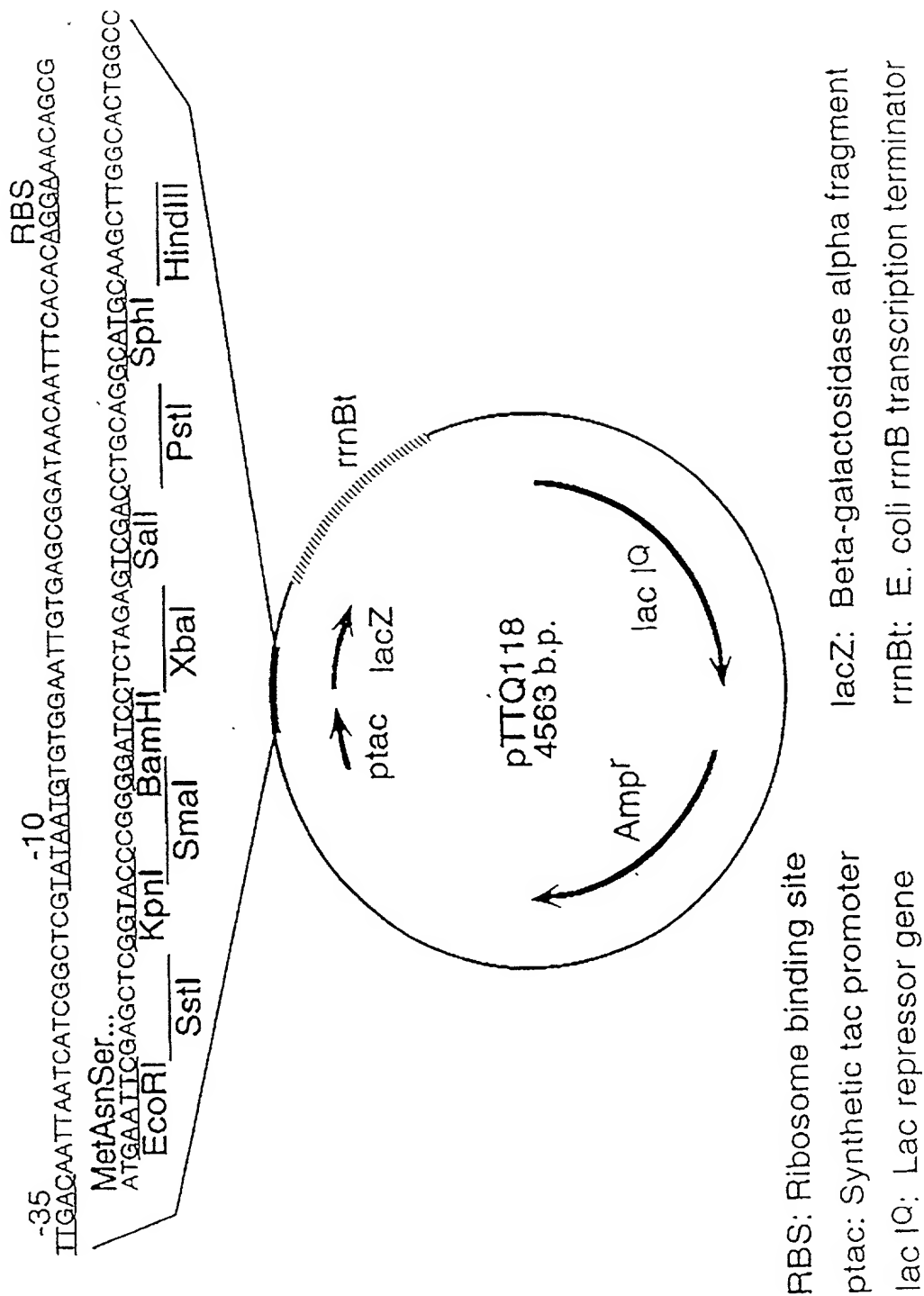
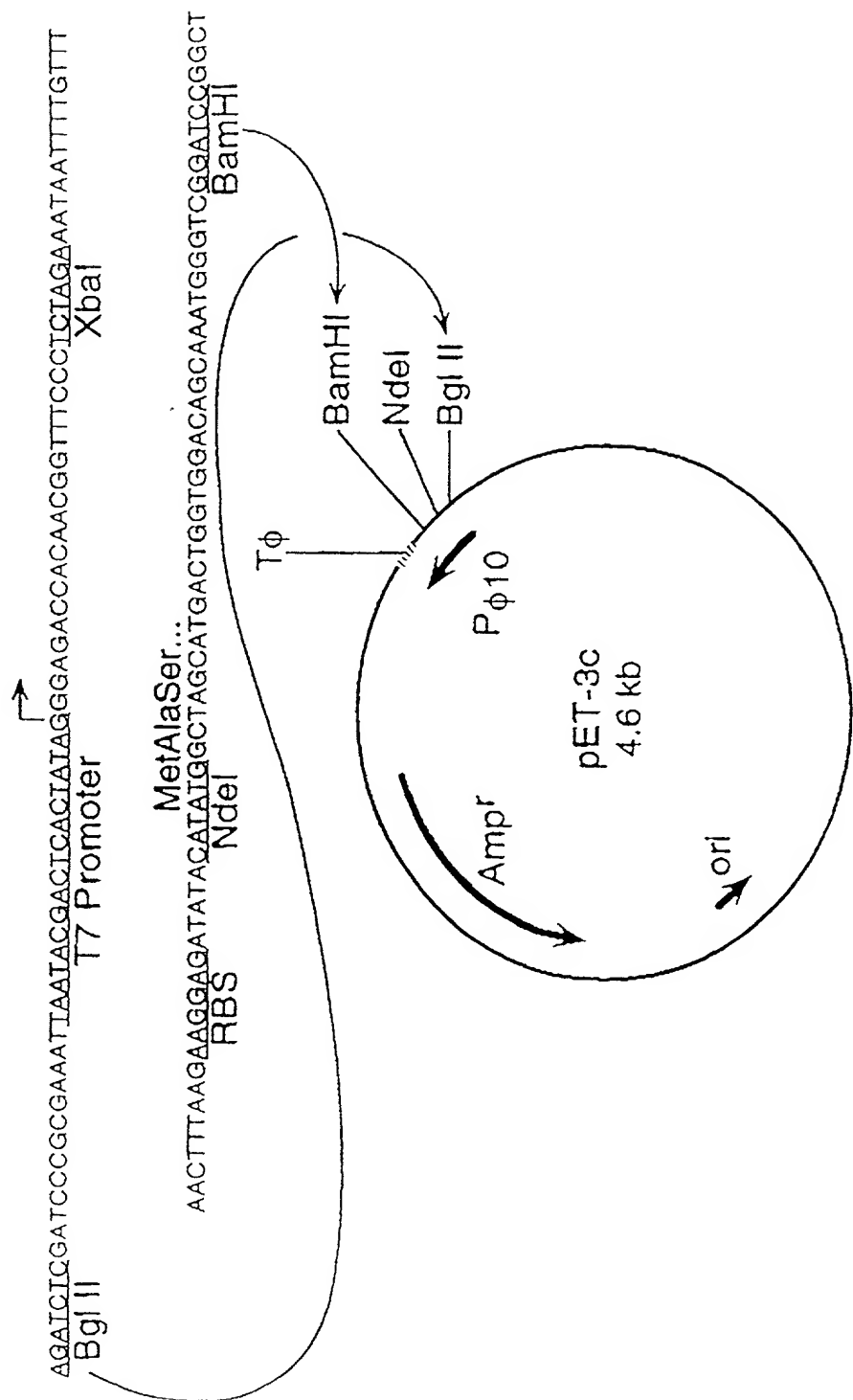


FIGURE 15



P_{φ10}: Bacteriophage T7 φ10 promoter
 T_φ: T7 φ Terminator
 RBS: Ribosome binding site

FIGURE 16

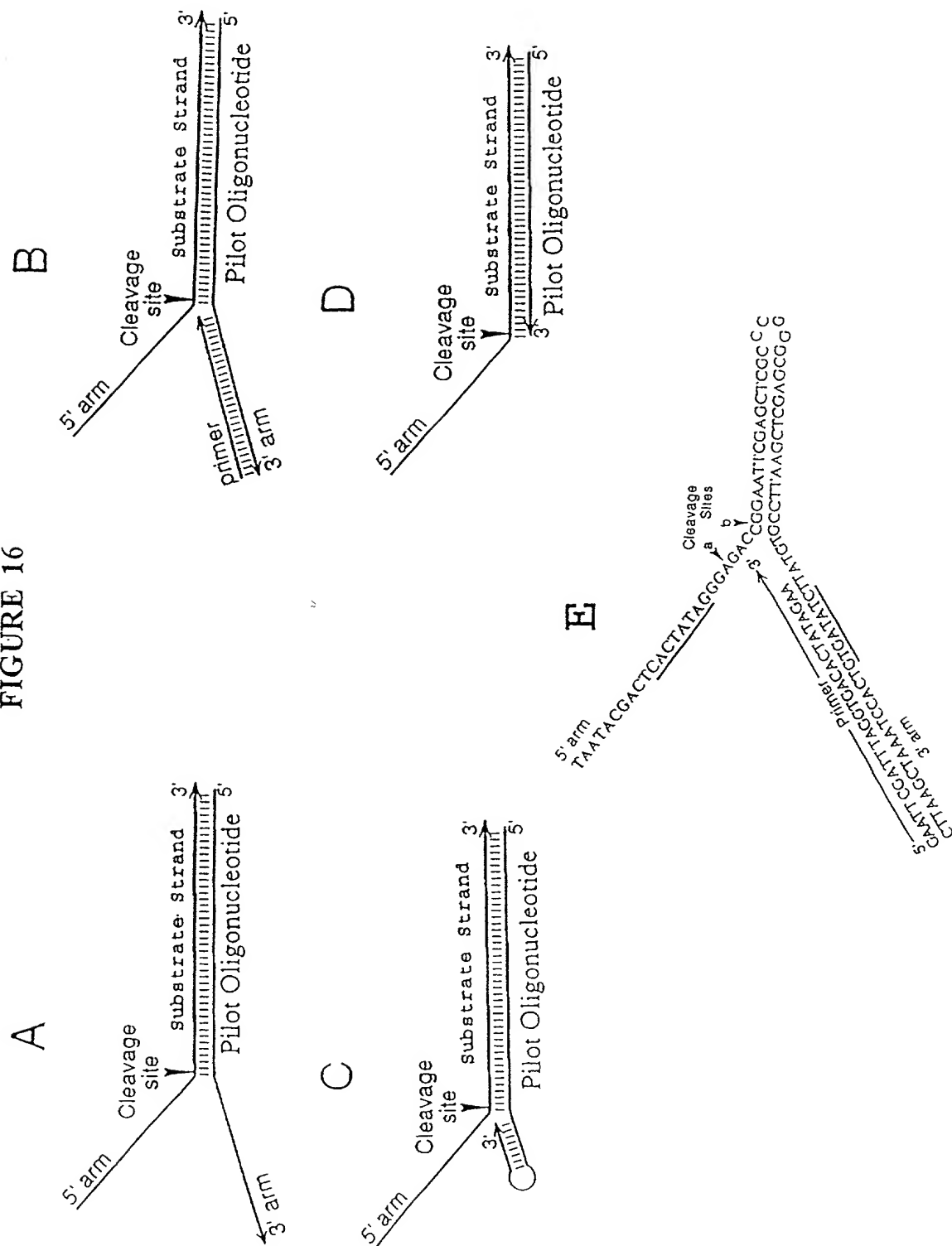


FIGURE 17

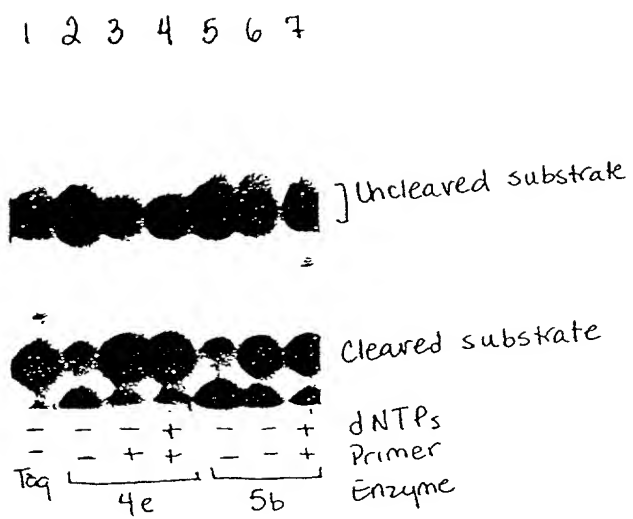


FIGURE 18

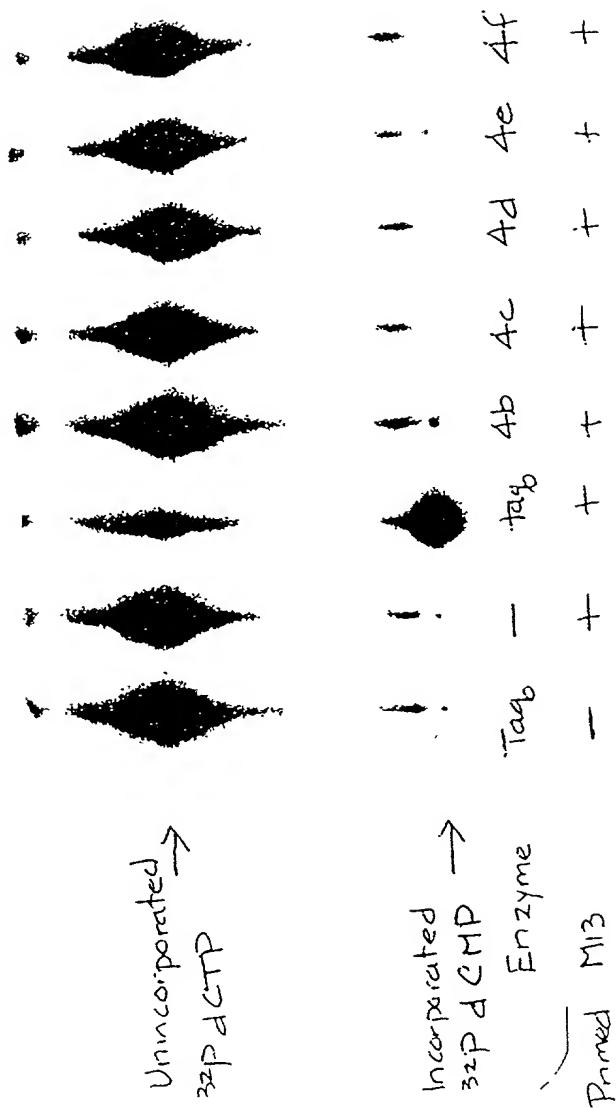
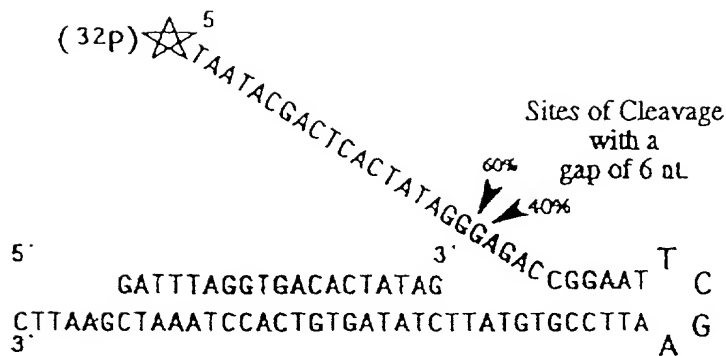


FIGURE 19



A




B

		"4d"		"4b"			
		No mutation		(2 pt. mutation)		Unmodified	
		Pol. Activity		small activity		DNAP Tag	
1	2	3	4	5	6	7	8
		C/A		T/A		T/A	
		+		-		+	

ATP

84 nt —  ← hairpin test molecule
 ← conversion to double stranded.
 (complete extension of primer)

desired product
 21 nuc.

 Multiple bands caused by polymerization

↑ some aberrant cleavage with "4b" because of residual polymerase activity.

DOE

5' --CGGACGAACAAGCGAGACAGCGACACAGI A
GTACC C
Tau CATGG A
CAAACACgacACAgCAgAgAgAACGGAGAA^ T
3'

A-Hairpin

Predicted cleavage sites

5' GTTTCGcctgTGTcGTcTcTcTTGCTCTT! A
GTACC T
Alpha CATGG G
---CTGCTGTTCGCTCTGTCTCGCTGTGTC^ T
3'

T-Hairpin

Sequence of alpha primer:

C

$$\begin{array}{rcl}
 & 5' & \text{ACACAGI} & \text{A} \\
 & & \text{GTACC} & \text{C} \\
 \text{"Tau"} & \longrightarrow & \text{CATGG} & \text{A} \\
 3' & \text{CAAAGACgacACAGcAGAgAgAACGGACAA} & & \text{T}
 \end{array}$$
 Cleaved A-Hairpin

$$\begin{array}{rcl}
 & 5' & \text{CTCTTTI} & \text{A} \\
 & & \text{GTACC} & \text{T} \\
 \text{"Alpha"} & \longrightarrow & \text{CATGG} & \text{G} \\
 3' & \text{CTGCTTGTTCCGCTCTGTGCGCTGTGTC} & & \text{T}
 \end{array}$$
 Cleaved T-Hairpin

Top = T-Hairpin
Bottom = A-Hairpin

Restriction sites (from left to right): BsmAI, Rsa I, Mnl I, Nla III, HgiC I, Nla IV, Rsa I, Kpn I, BsmAI.

Sequence (Top strand): GTTCTGCTGTGTCGTCCTCTCTCTGCTCTTGTACCACTGTGGTACCTGTGTCGCTGTCTCGCTTGTTCGTC

Sequence (Bottom strand): CAAAGACGACACAGCAGAGAGAAACGGAGAAACATGGTATCCATCGACACAGCGACAGAGCGAACAAGCAGGC

72

FIGURE 21

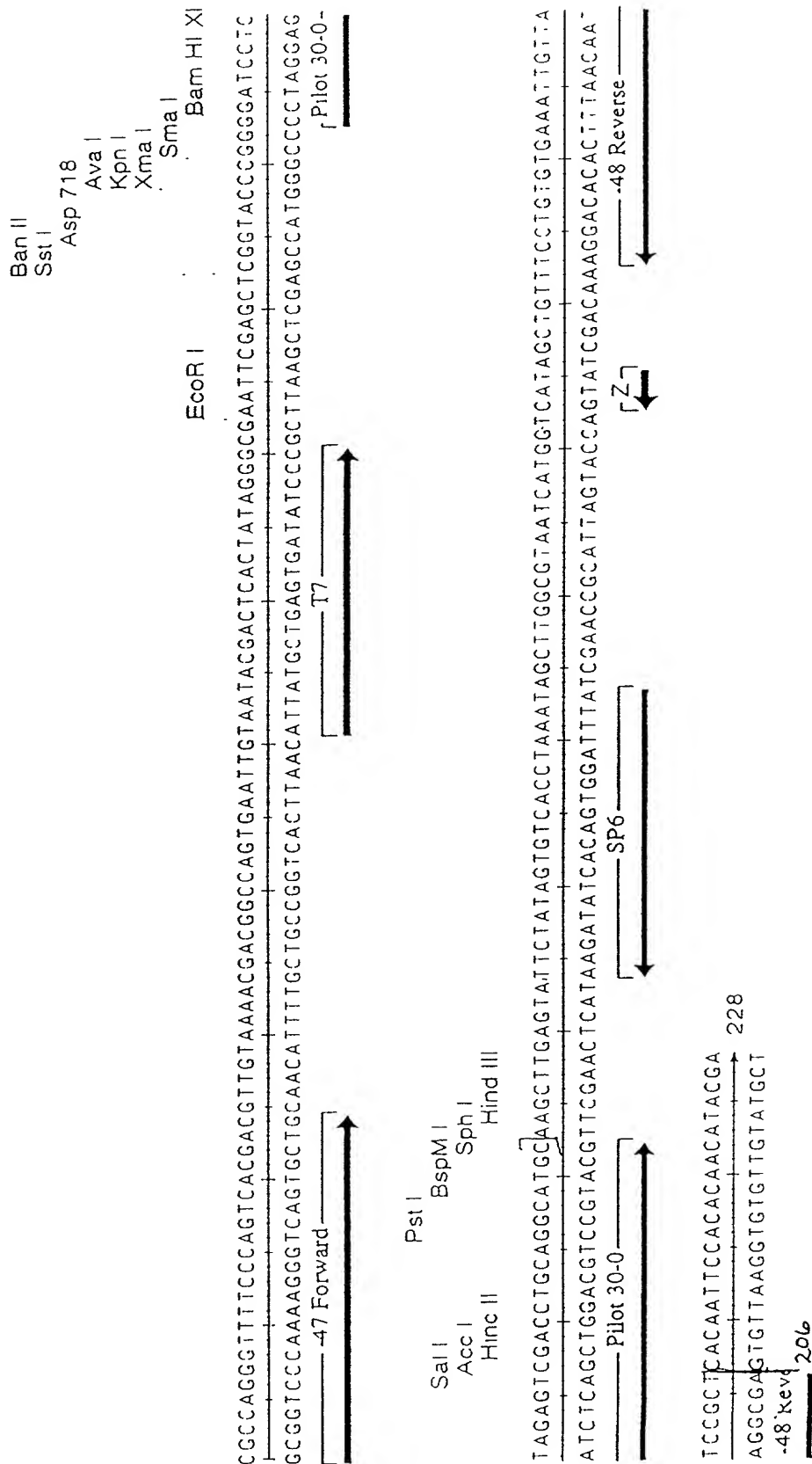


FIGURE 22A

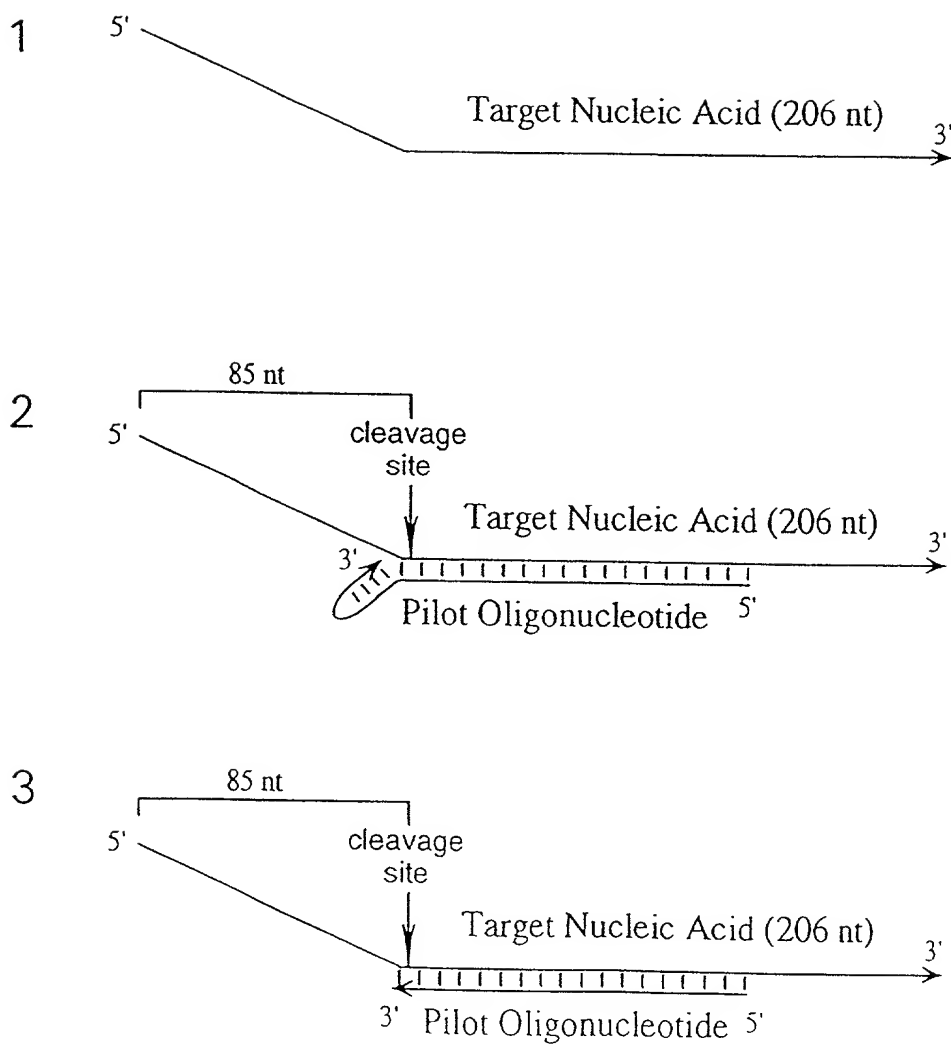


FIGURE 22B

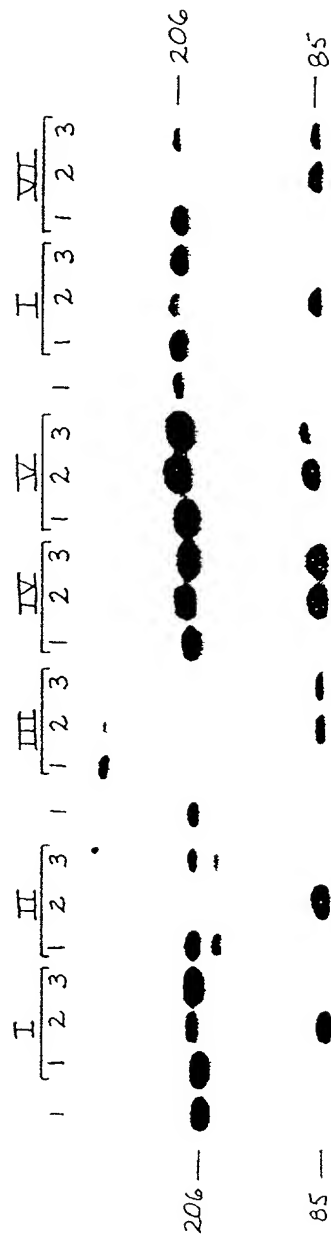


FIGURE 23

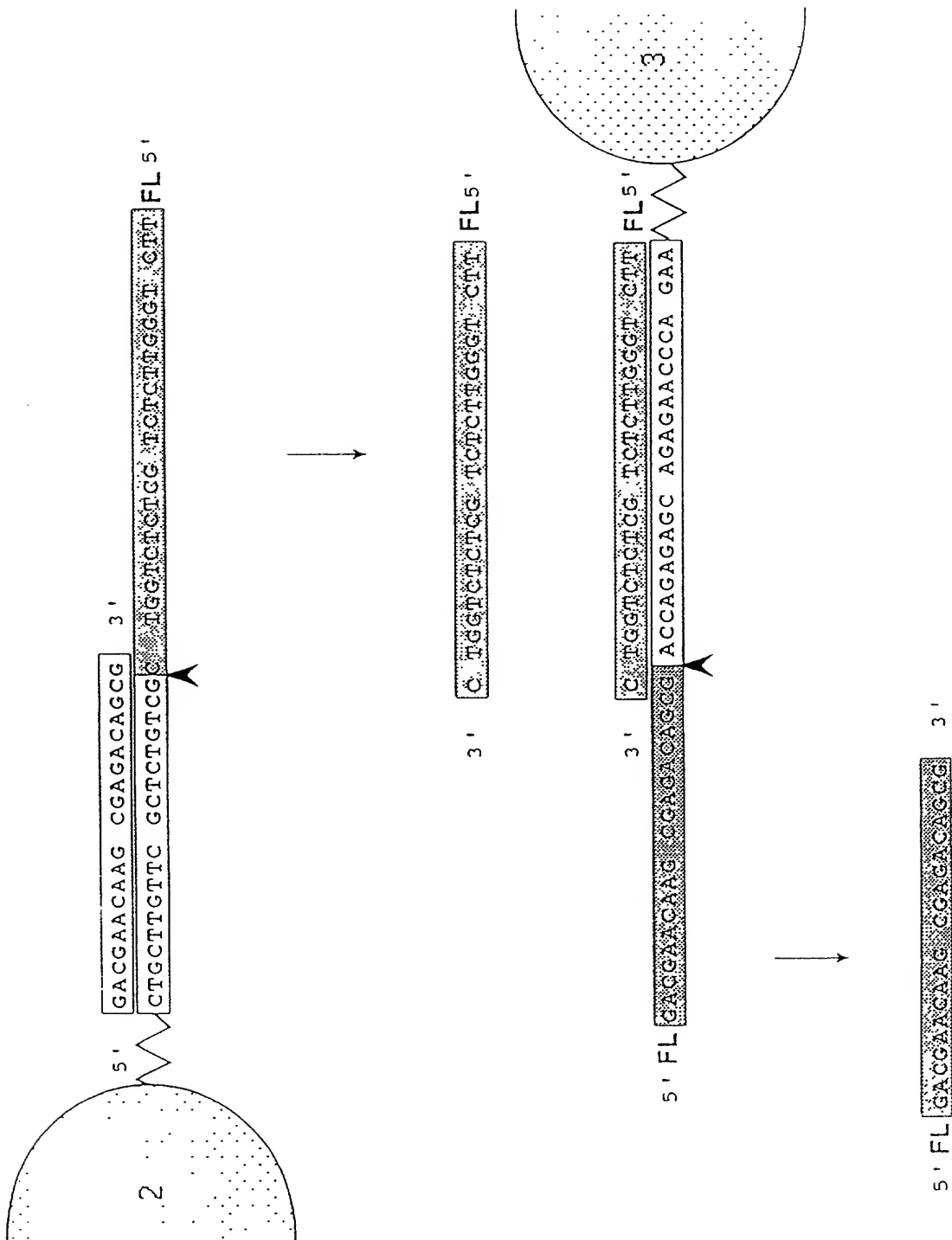


FIGURE 25

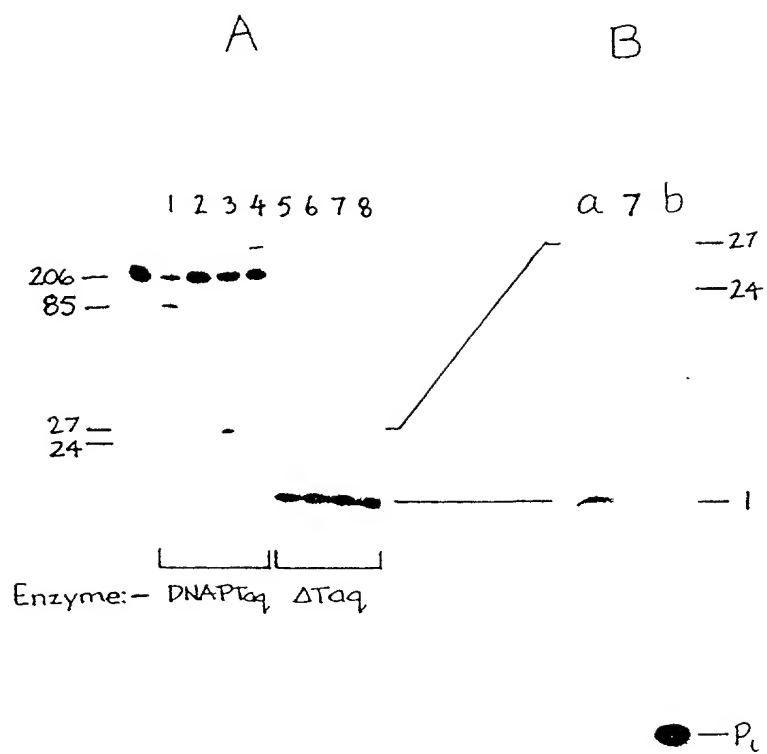
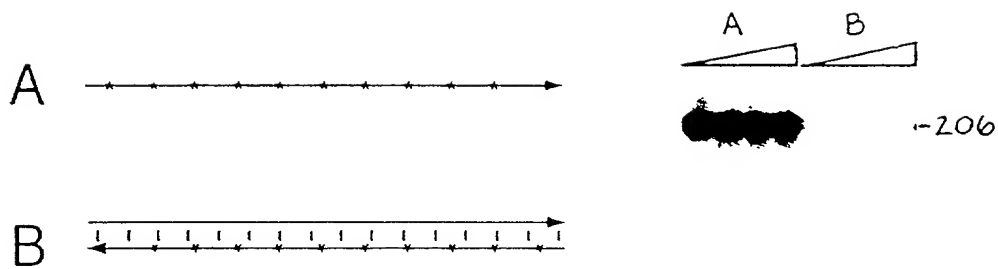
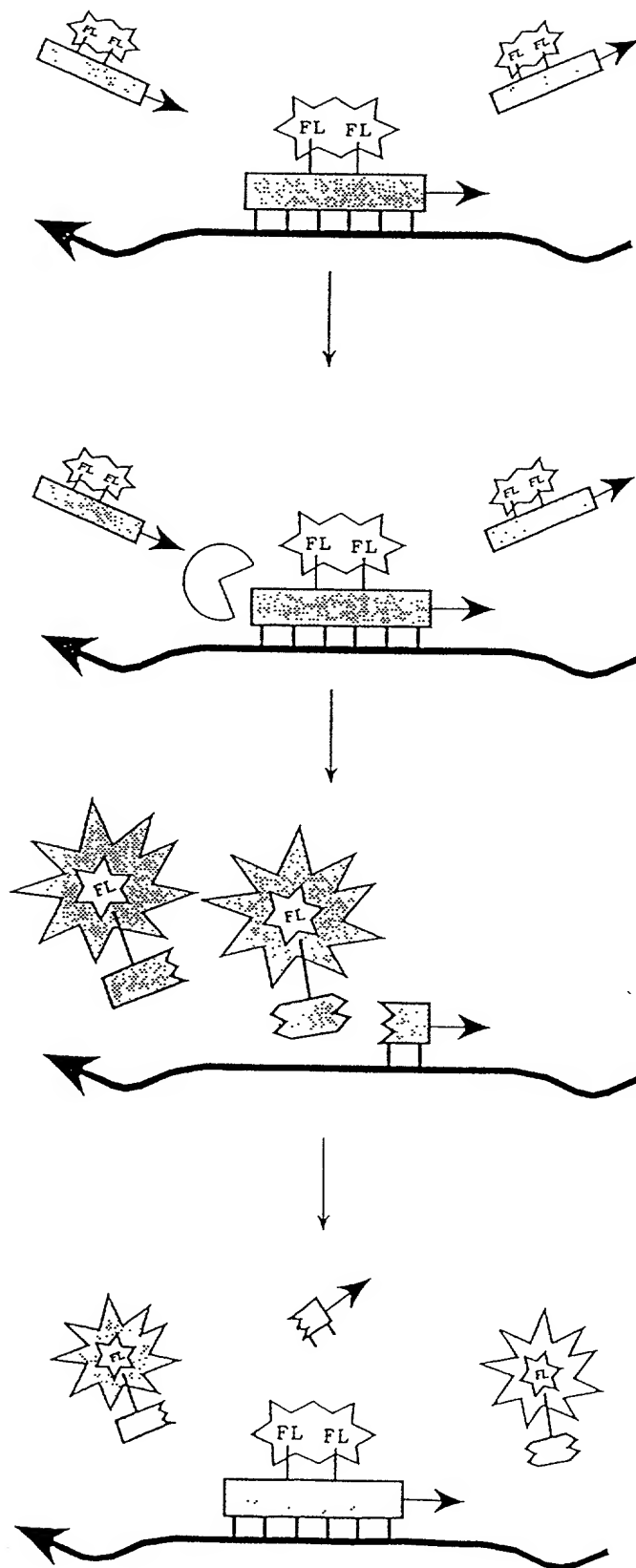


FIGURE 26



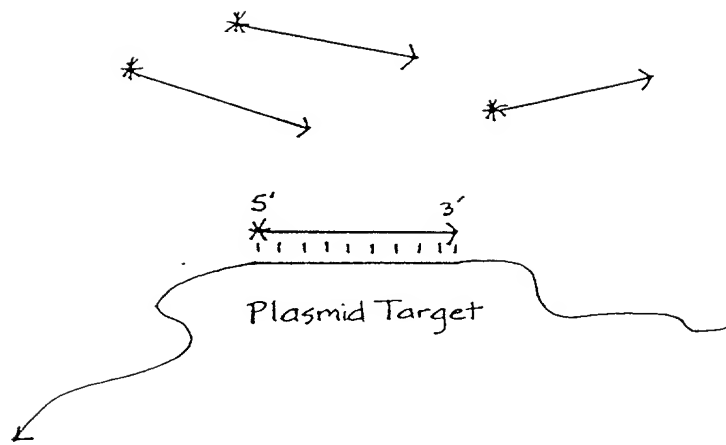
* = ^{32}P

FIGURE 27



10074323.024202

FIGURE 28A



* = ^{32}P 5' terminal phosphate

2025-02-24 10:00

FIGURE 28B

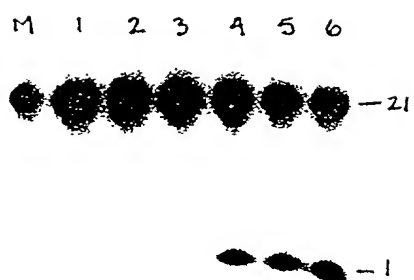


FIGURE 29

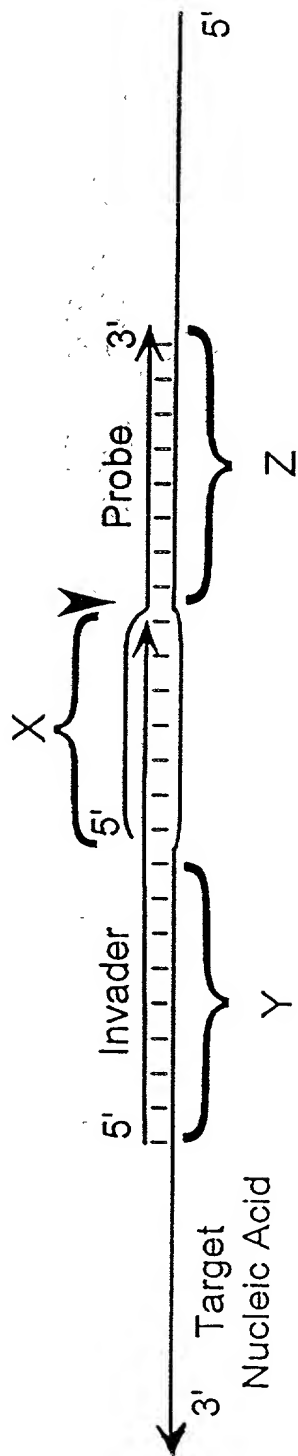


FIGURE 30

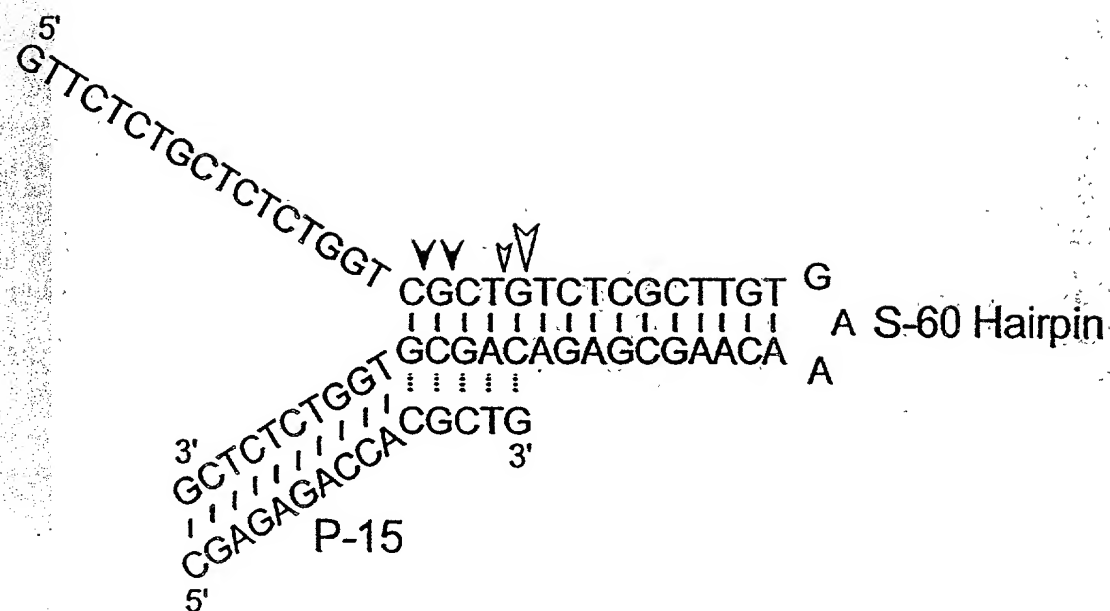
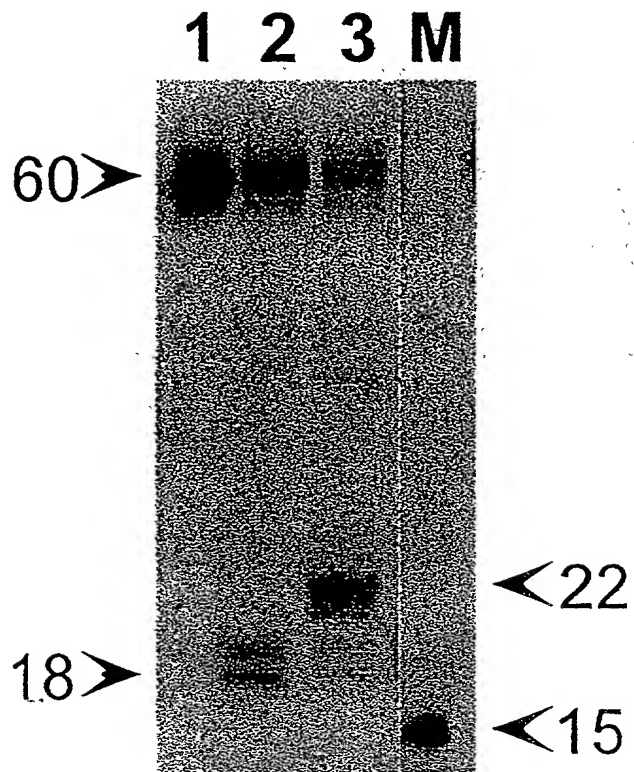
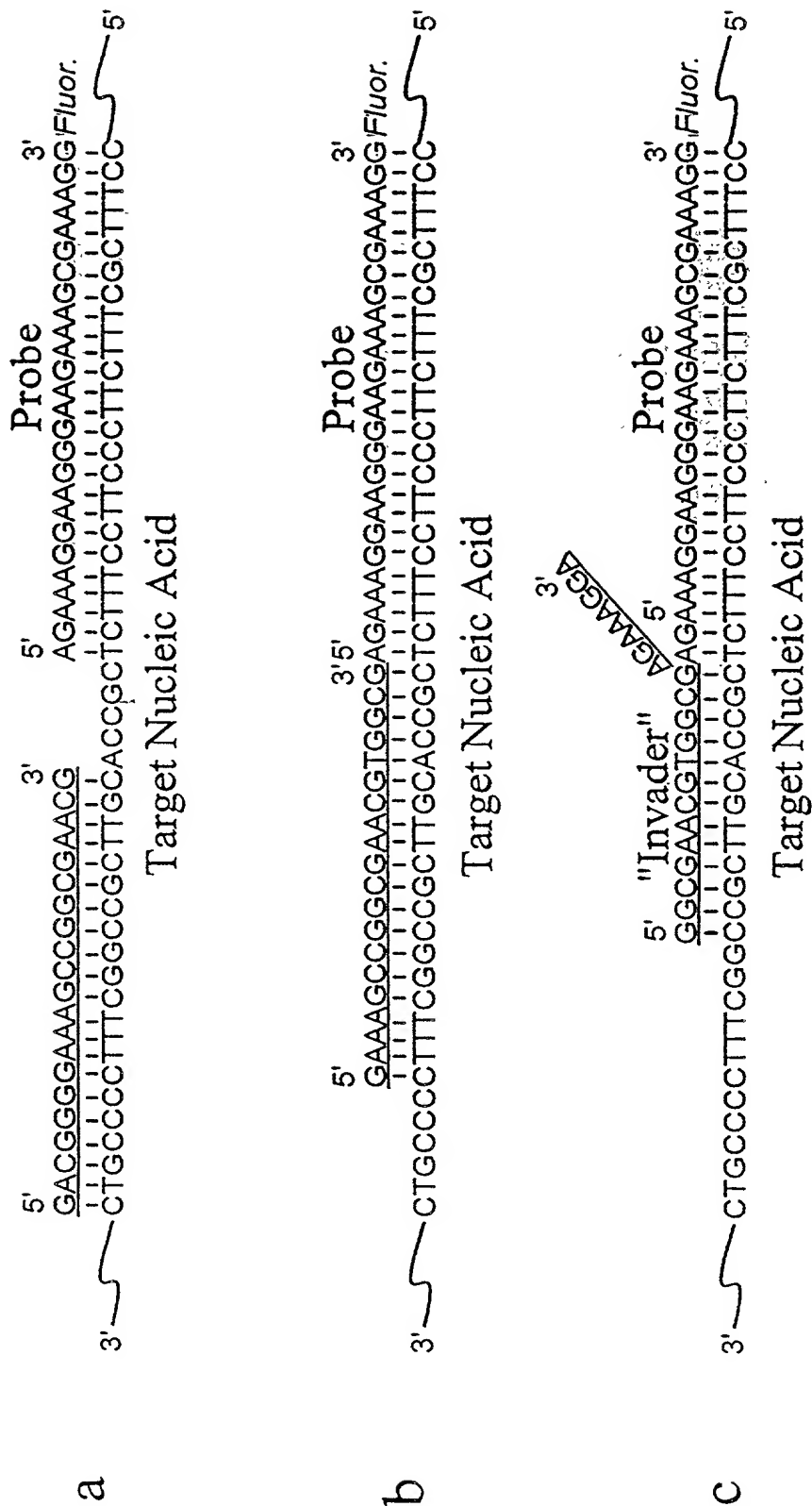


FIGURE 31



202120" 82E4/00T

FIGURE 32
202120" BEEH001



44

202120" 82E41001

8

7

6

5

4

3

2

1

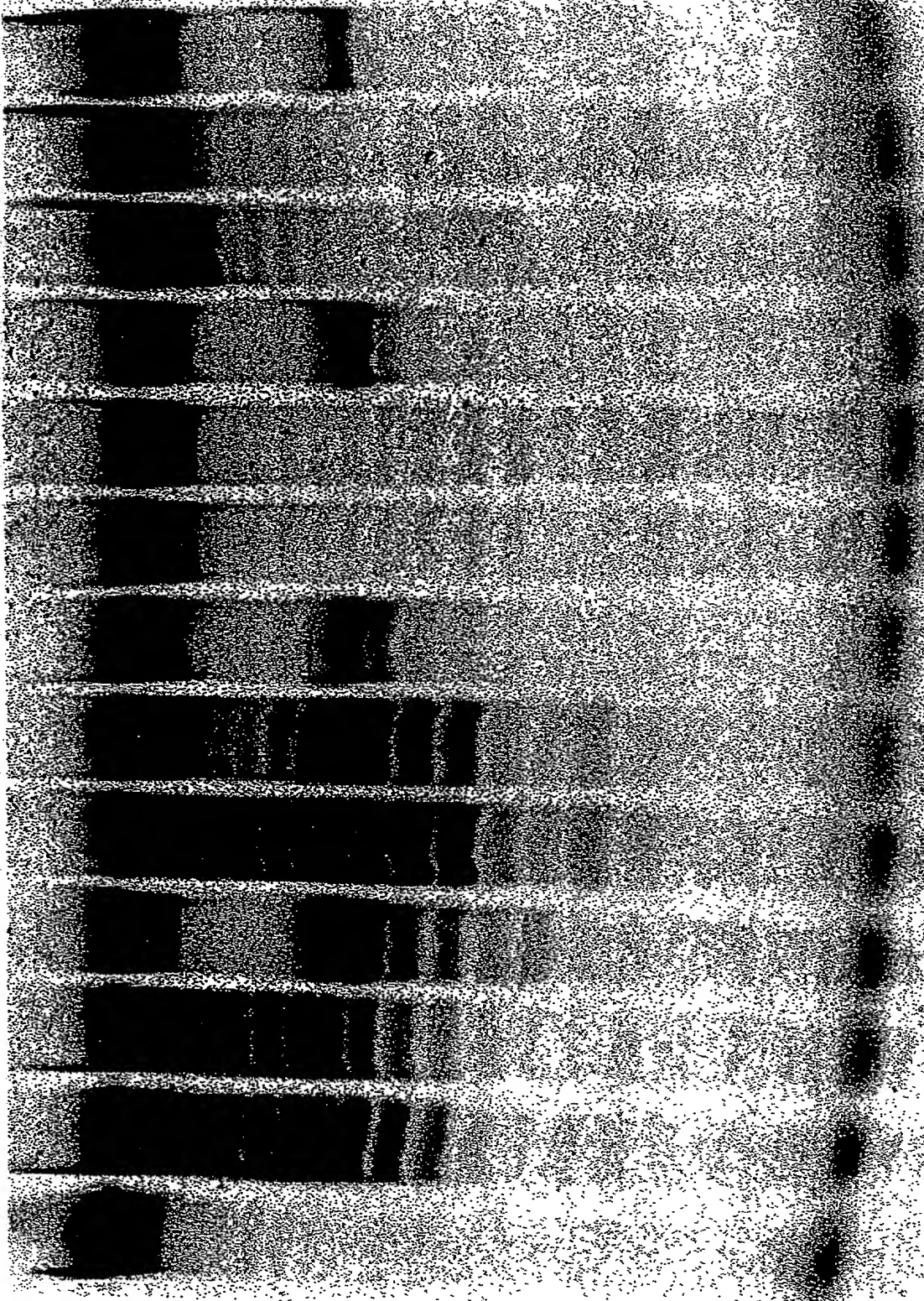
267

45

FIGURE 34

202120" 82E11/00T

M 1 2 3 4 5 6 7 8 9 10 11 12

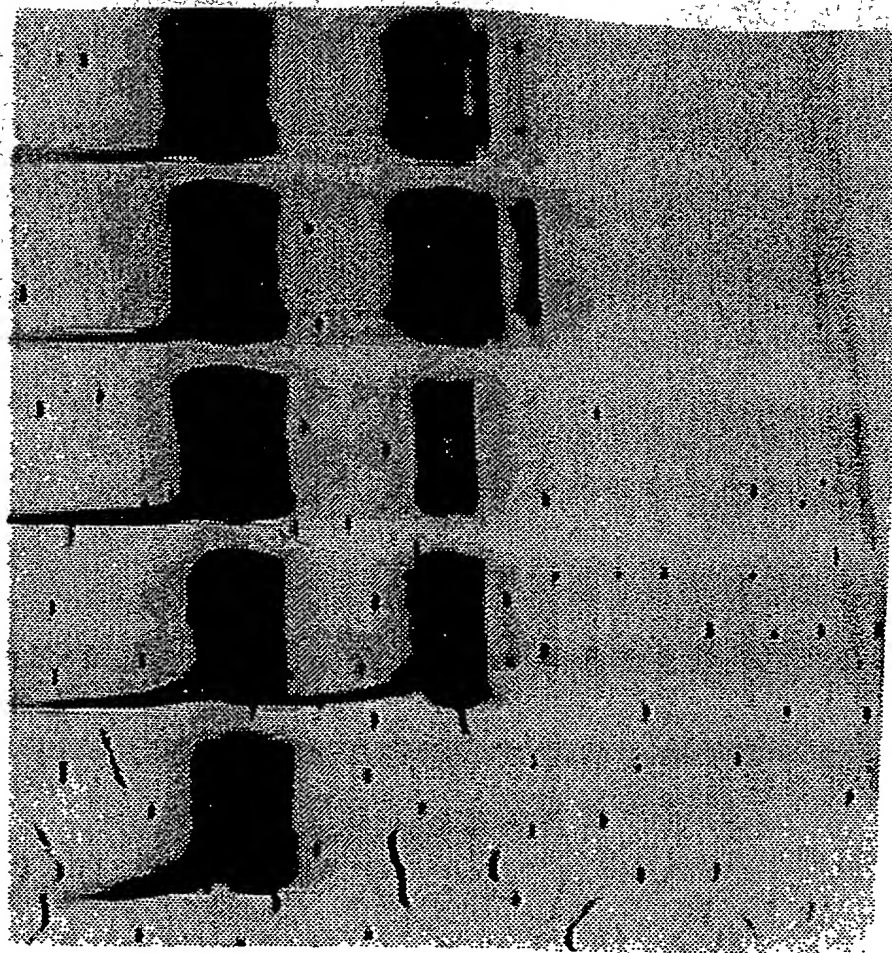


26

46

202120" B2E4/001

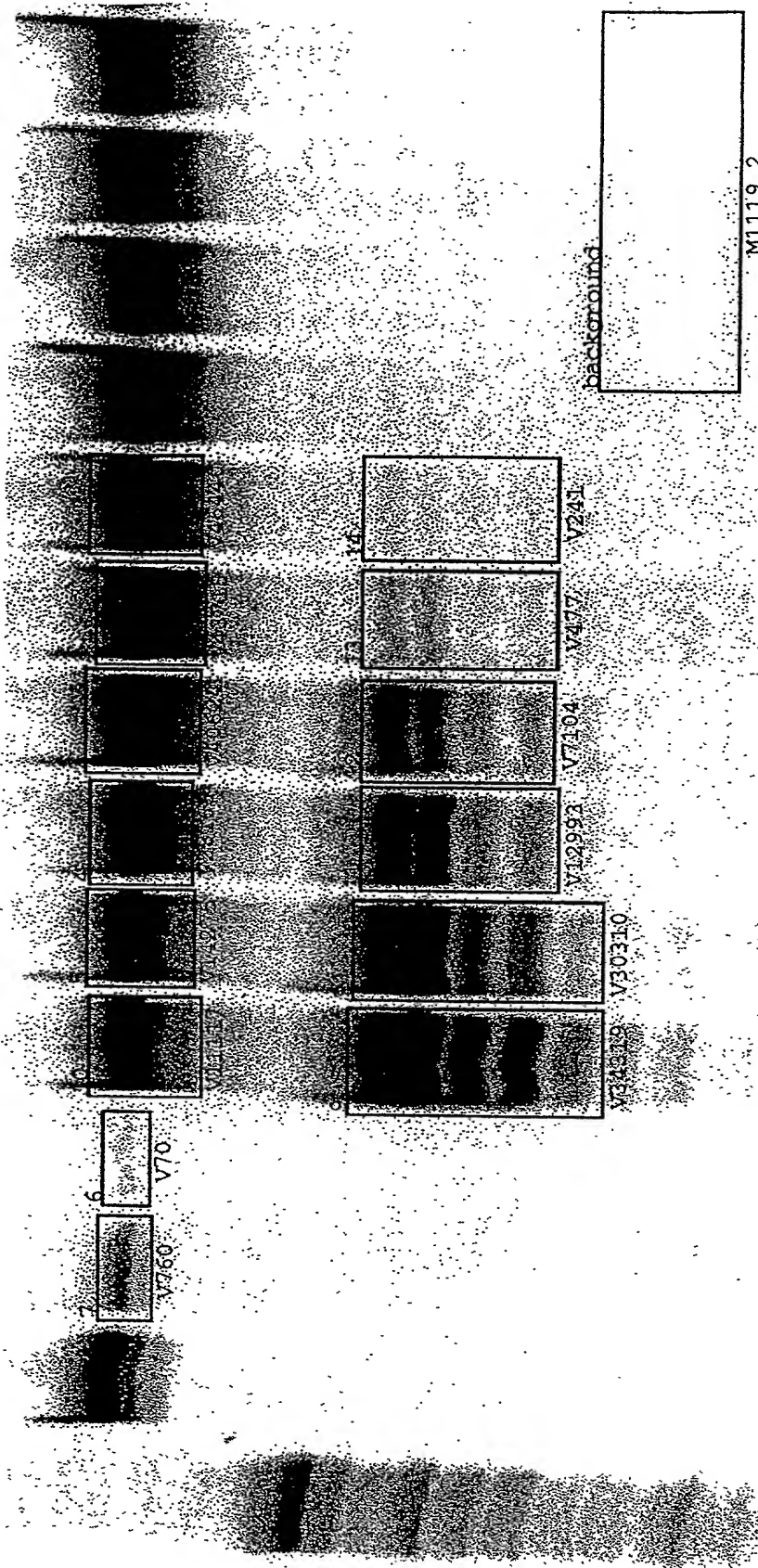
1 2 3 4 5



26

FIGURE 36
202120" B2E1/001

M 1 2 3 4 5 6 7 8 9 10 11 12 13



202120" B2E42001

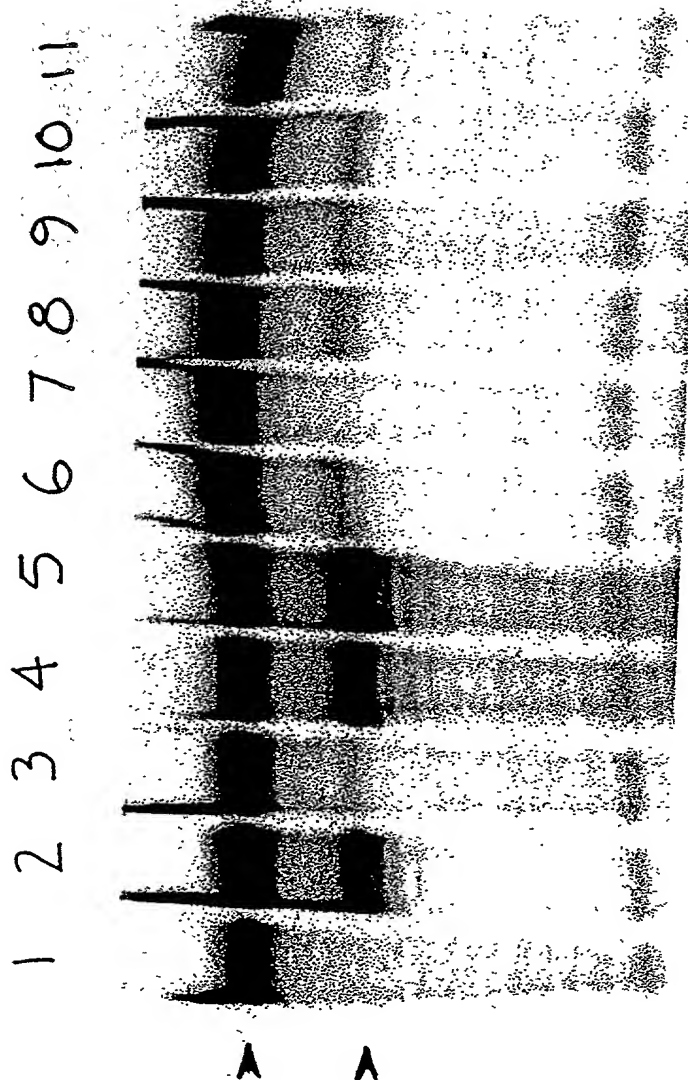


FIGURE 38 E2E4/001

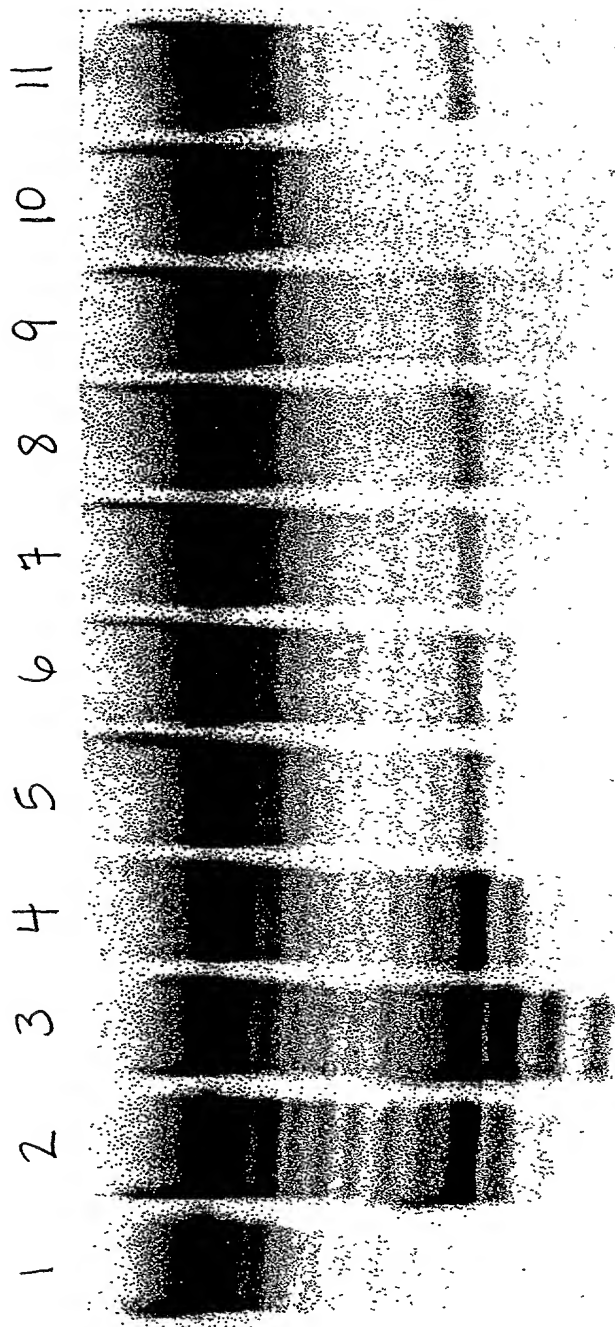


FIGURE 39

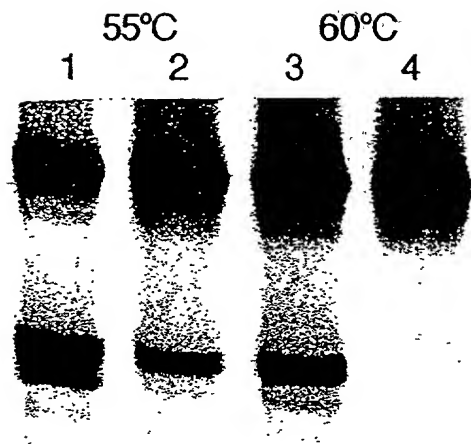


FIGURE 40

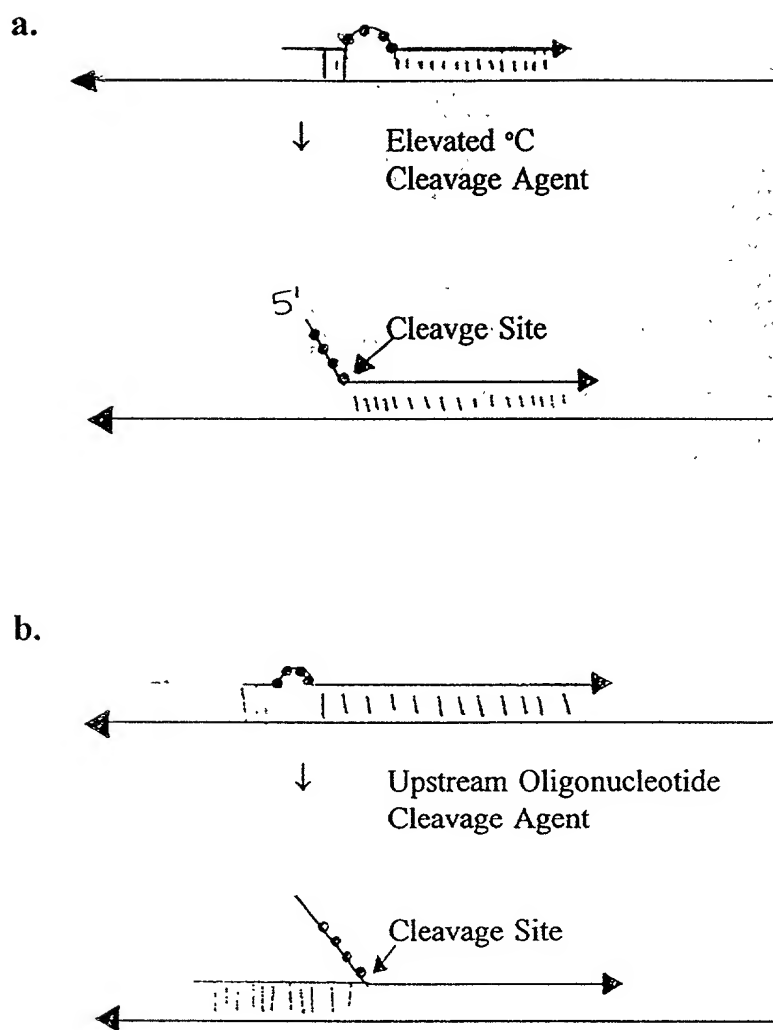


FIGURE 41

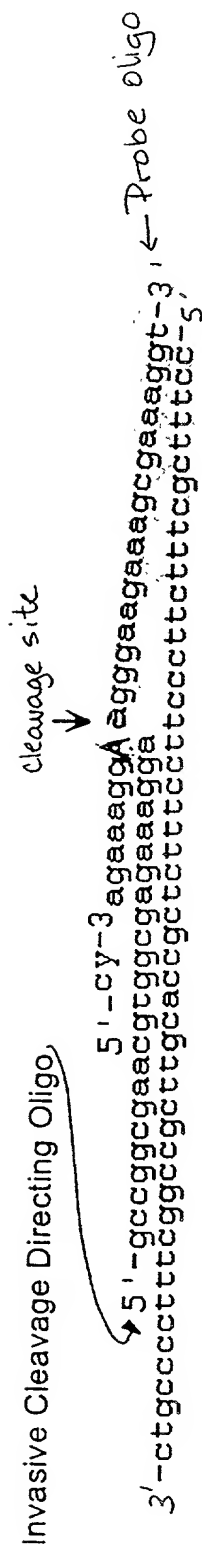
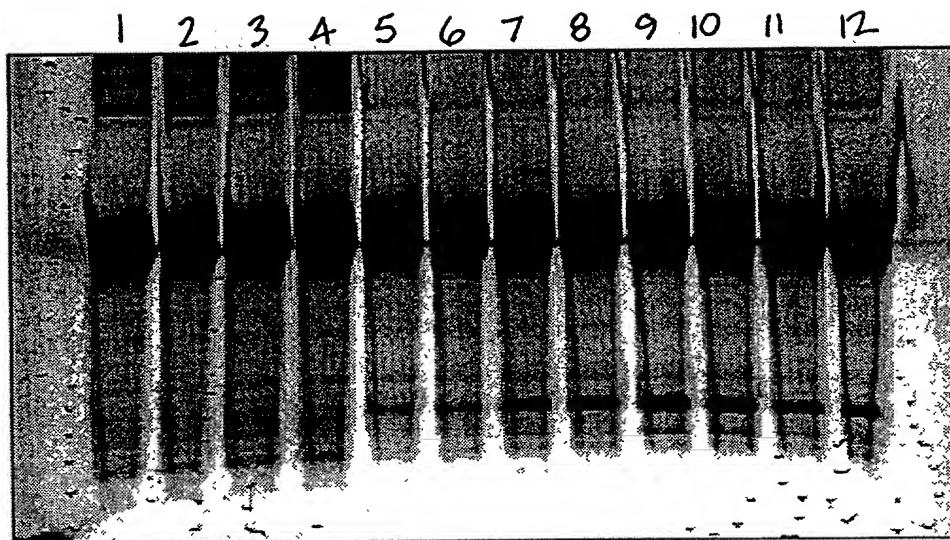
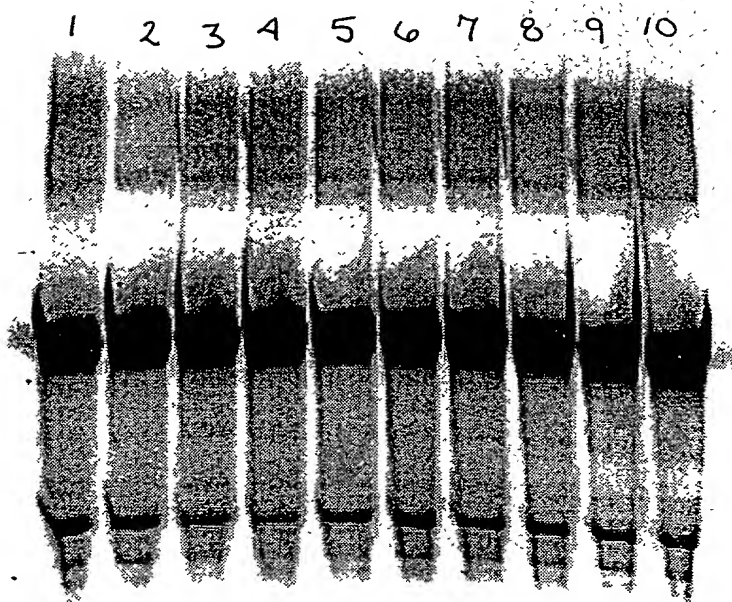


FIGURE 42



202T20" B2E4001

FIGURE 43



SS

FIGURE 44

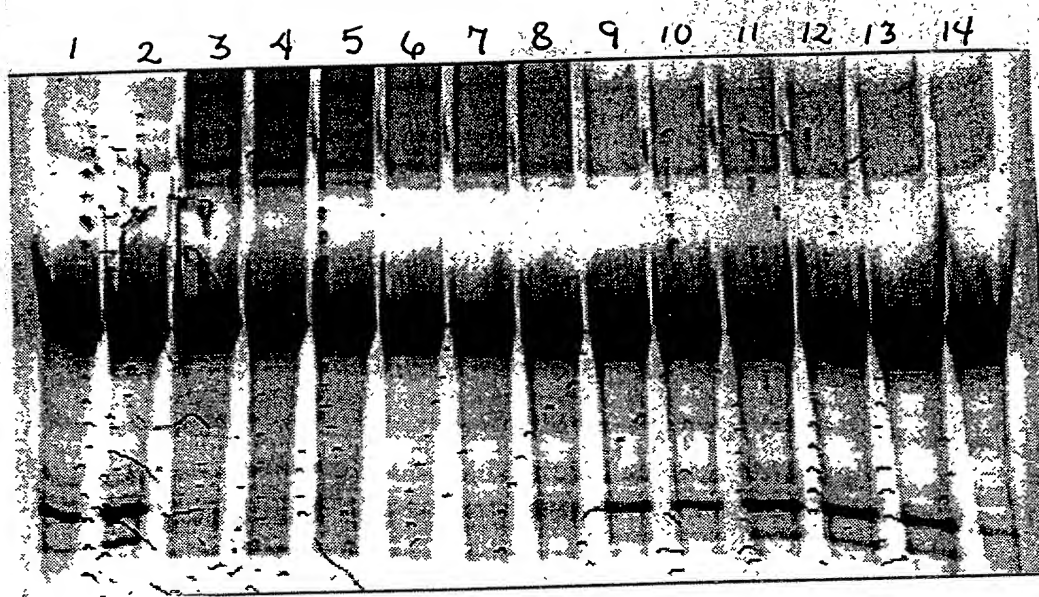


FIGURE 45

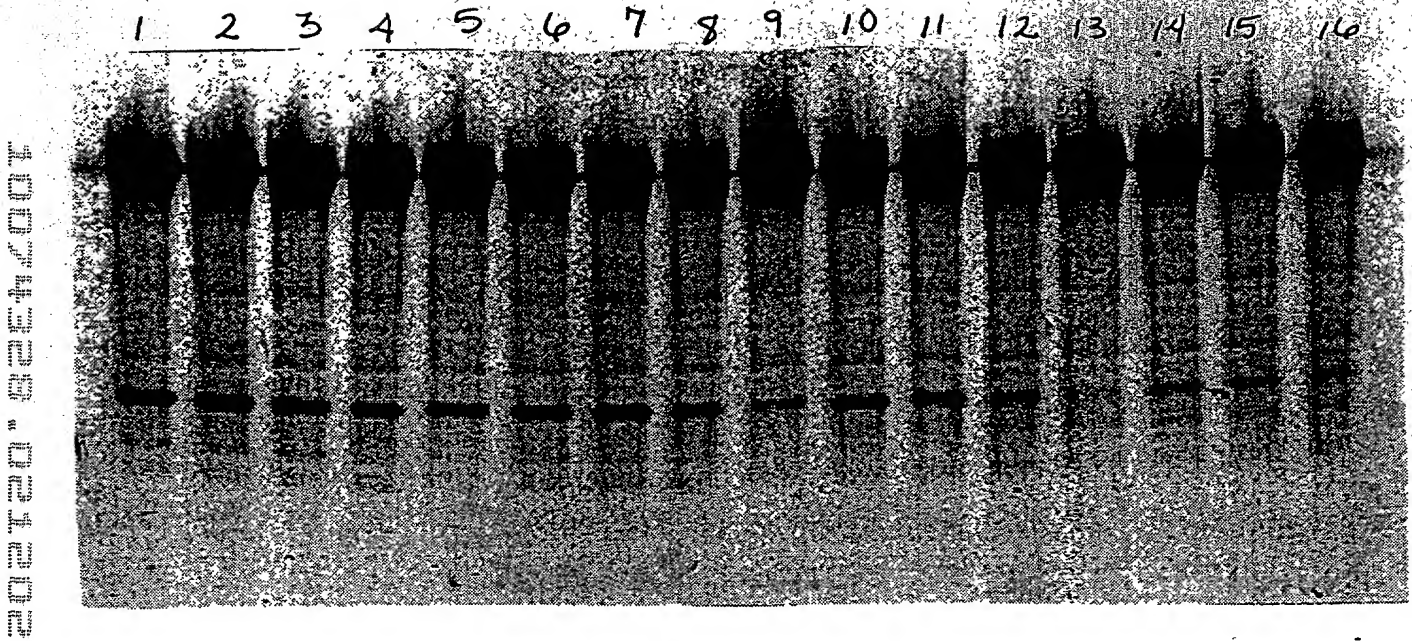
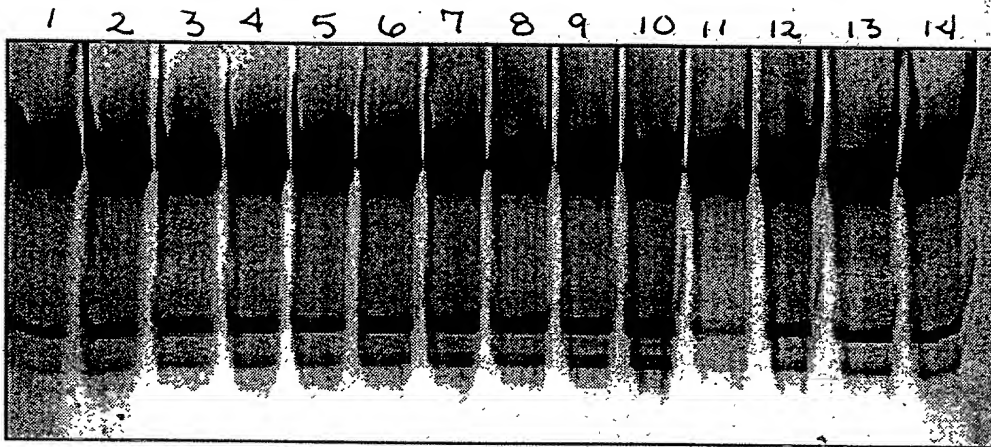


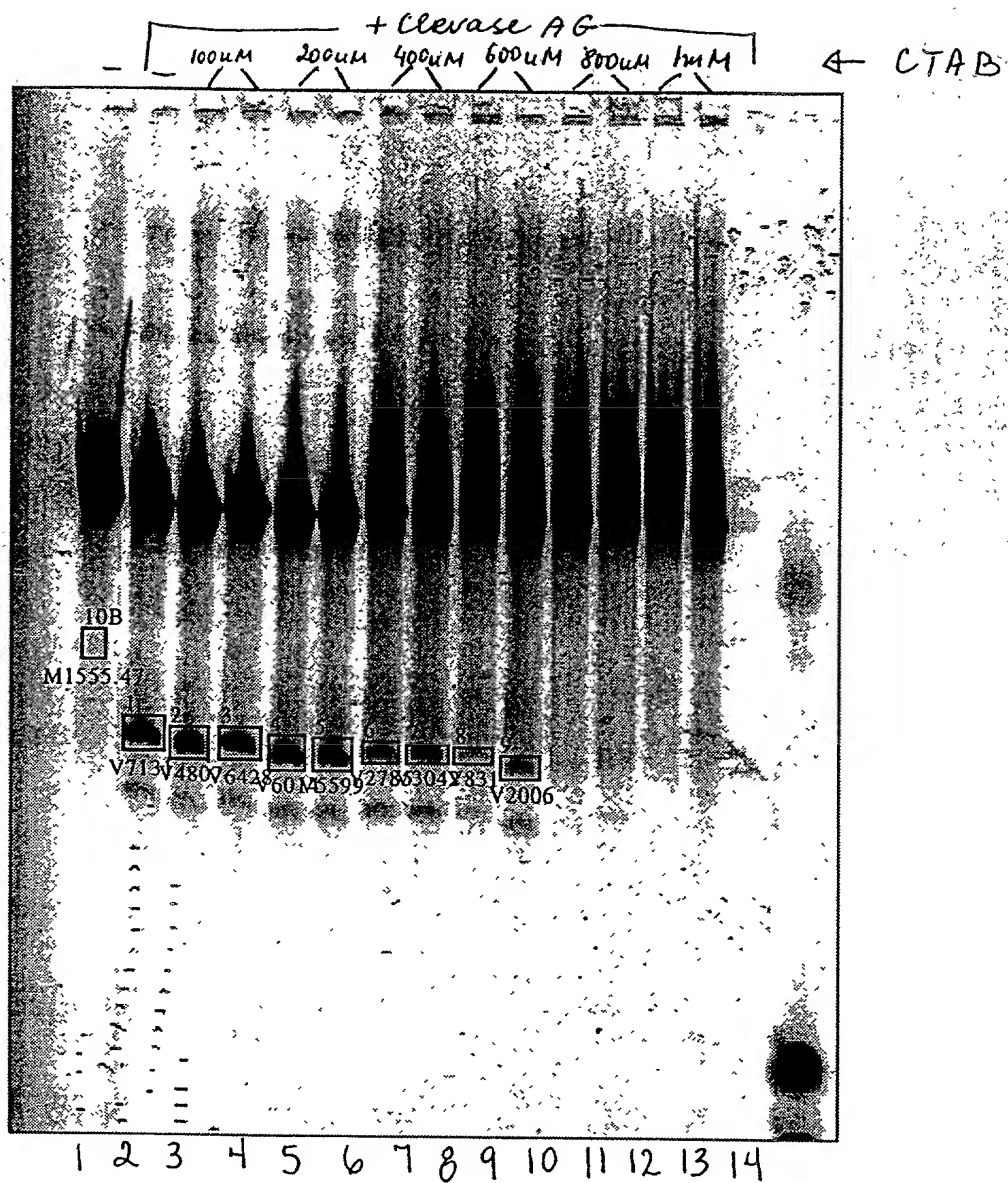
FIGURE 46



10074323.024203

58

FIGURE 47



59

202120" 32E4200T

FIGURE 48

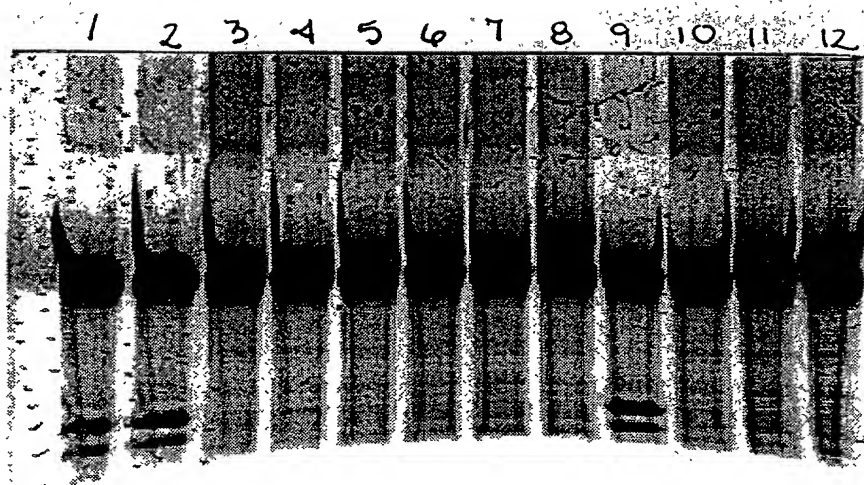
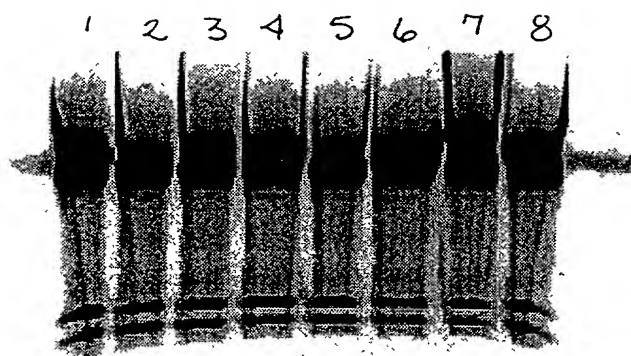
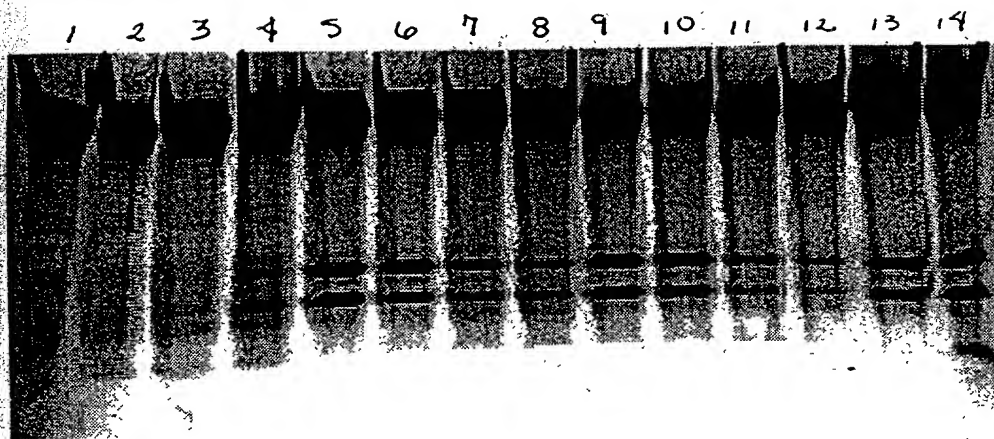


FIGURE 49



10074328 .024202
202120" BEEH/001

FIGURE 50



202120 32E4/001

FIGURE 51

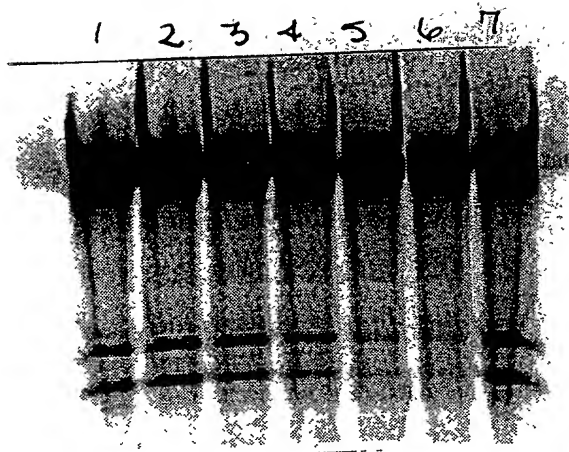
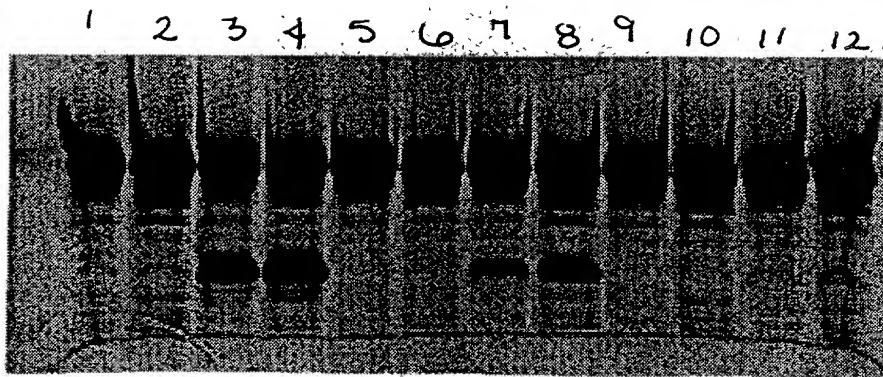


FIGURE 52

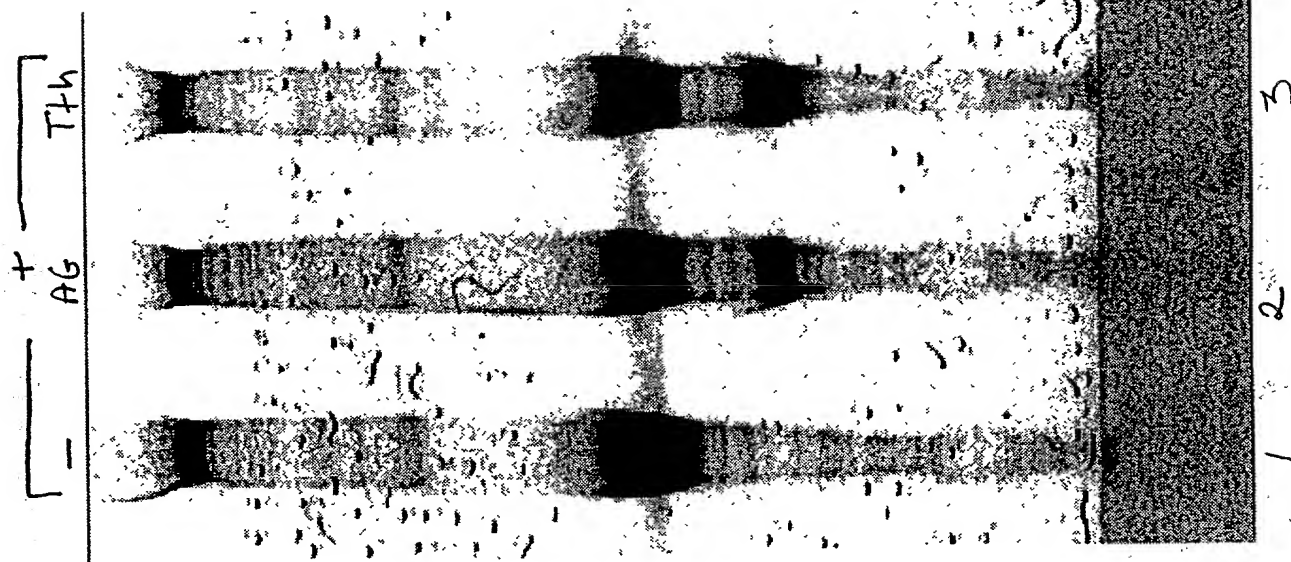


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64

FIGURE 53 ETH 001

a



TARGET RNA →

UNCLEAVED
PROBE →

CLEAVED
PROBE →

65

b

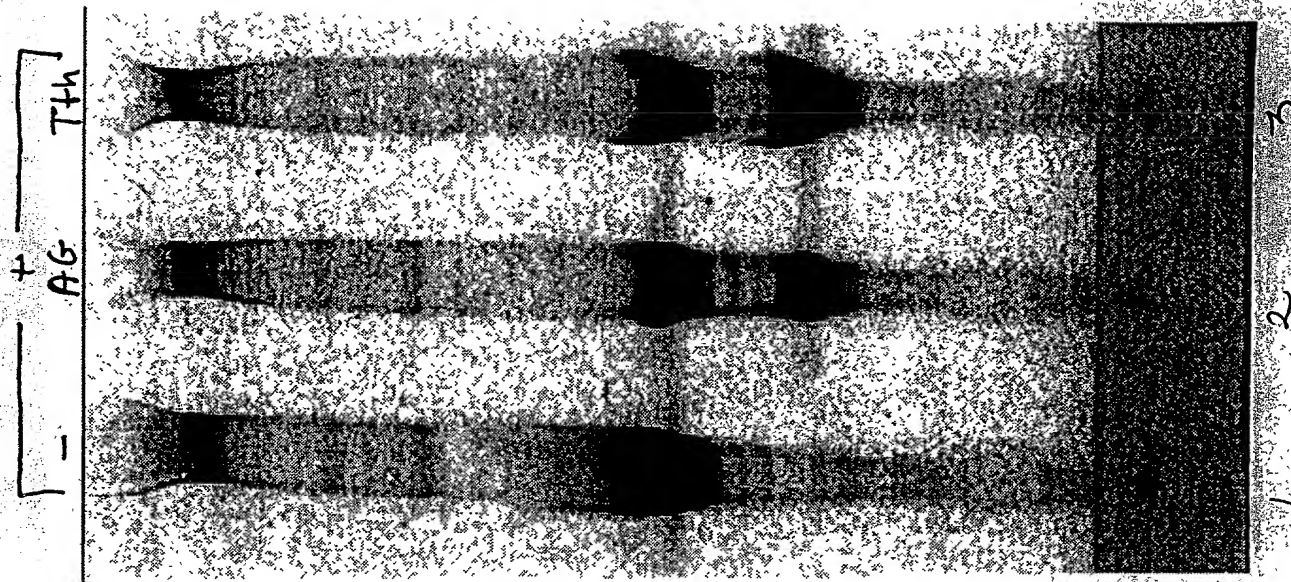


FIGURE 54

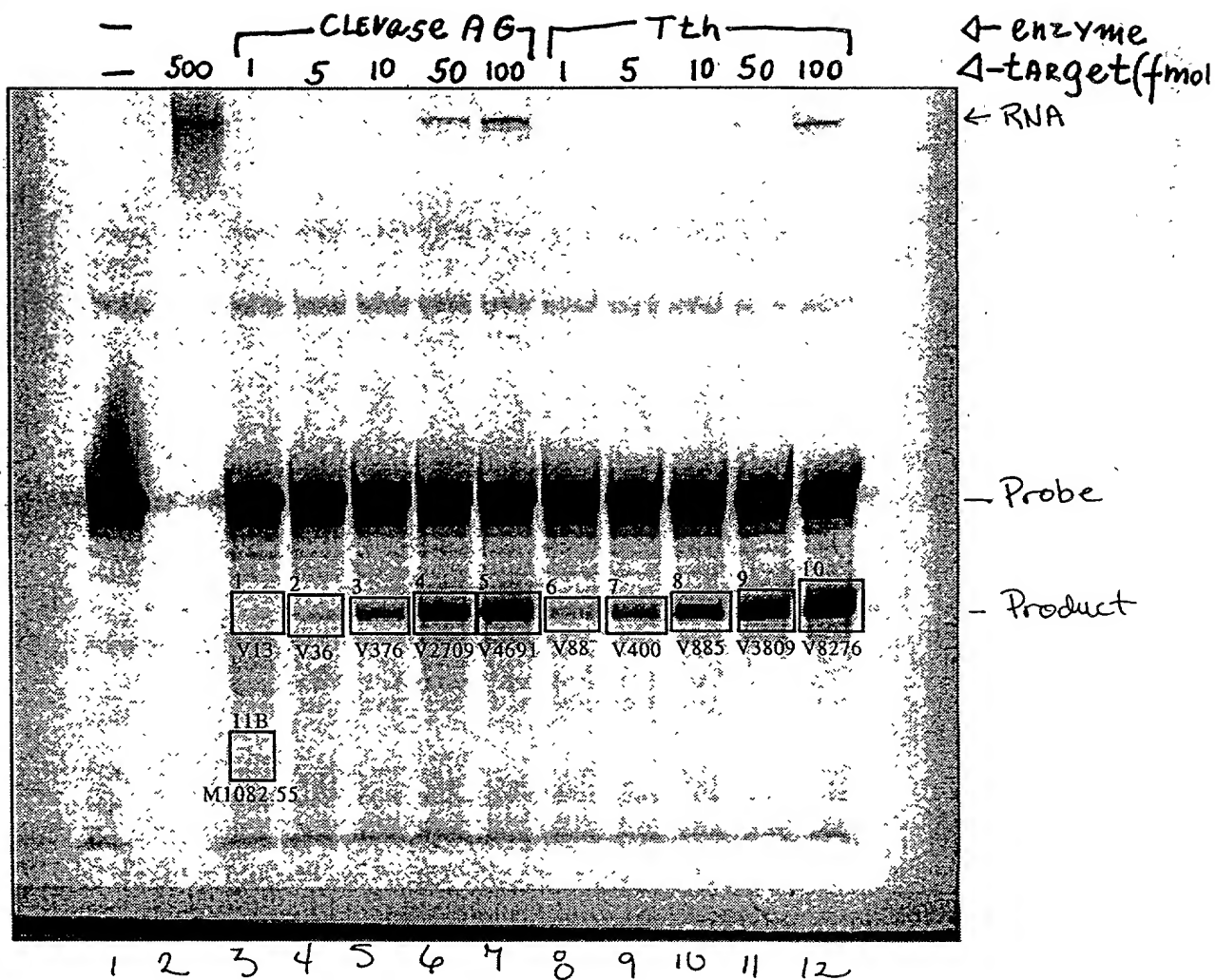


FIGURE 55

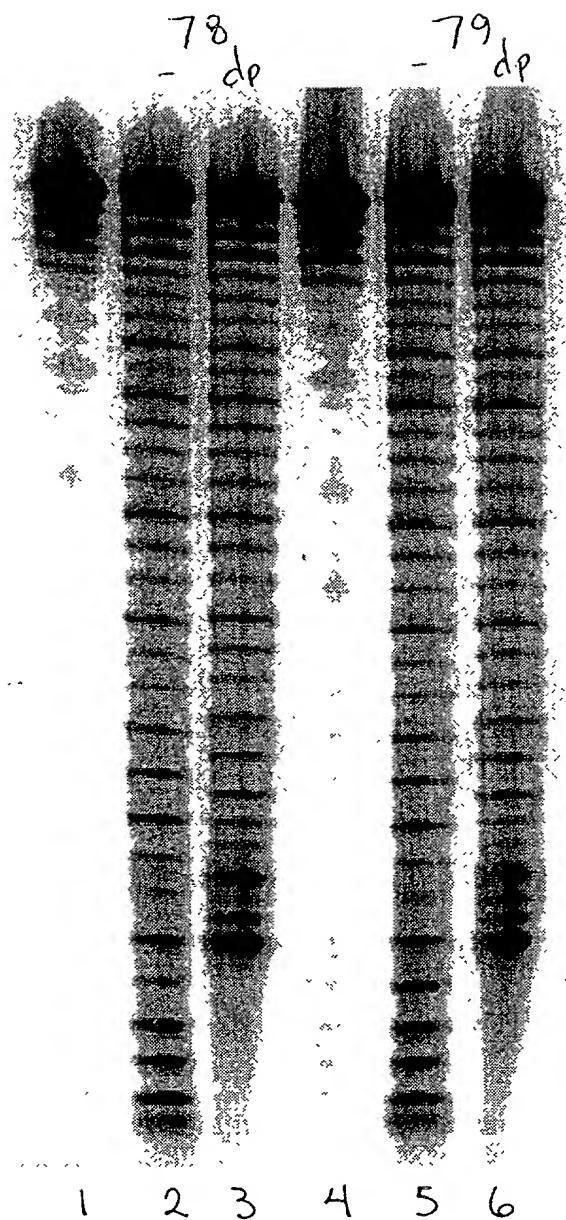
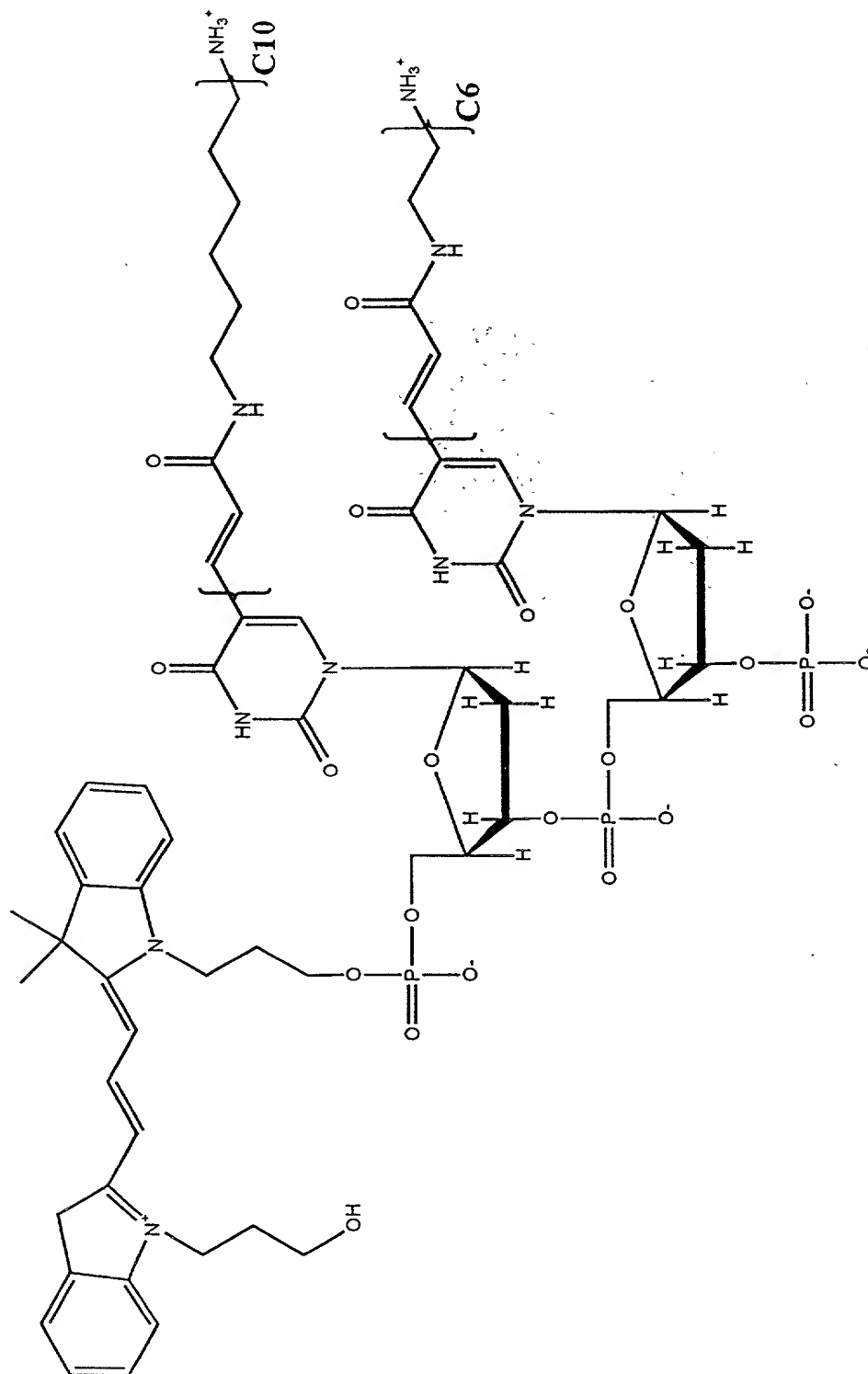
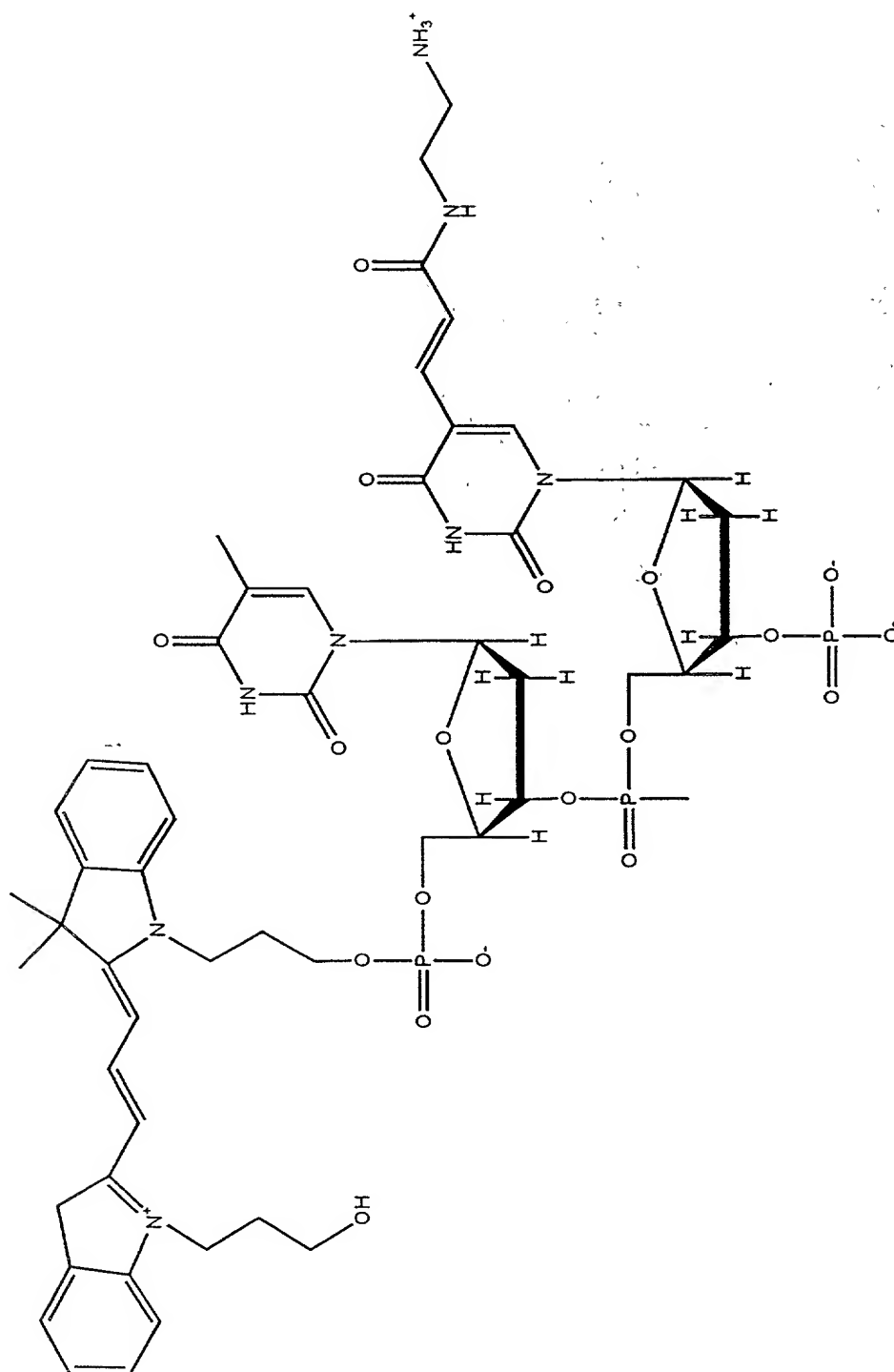
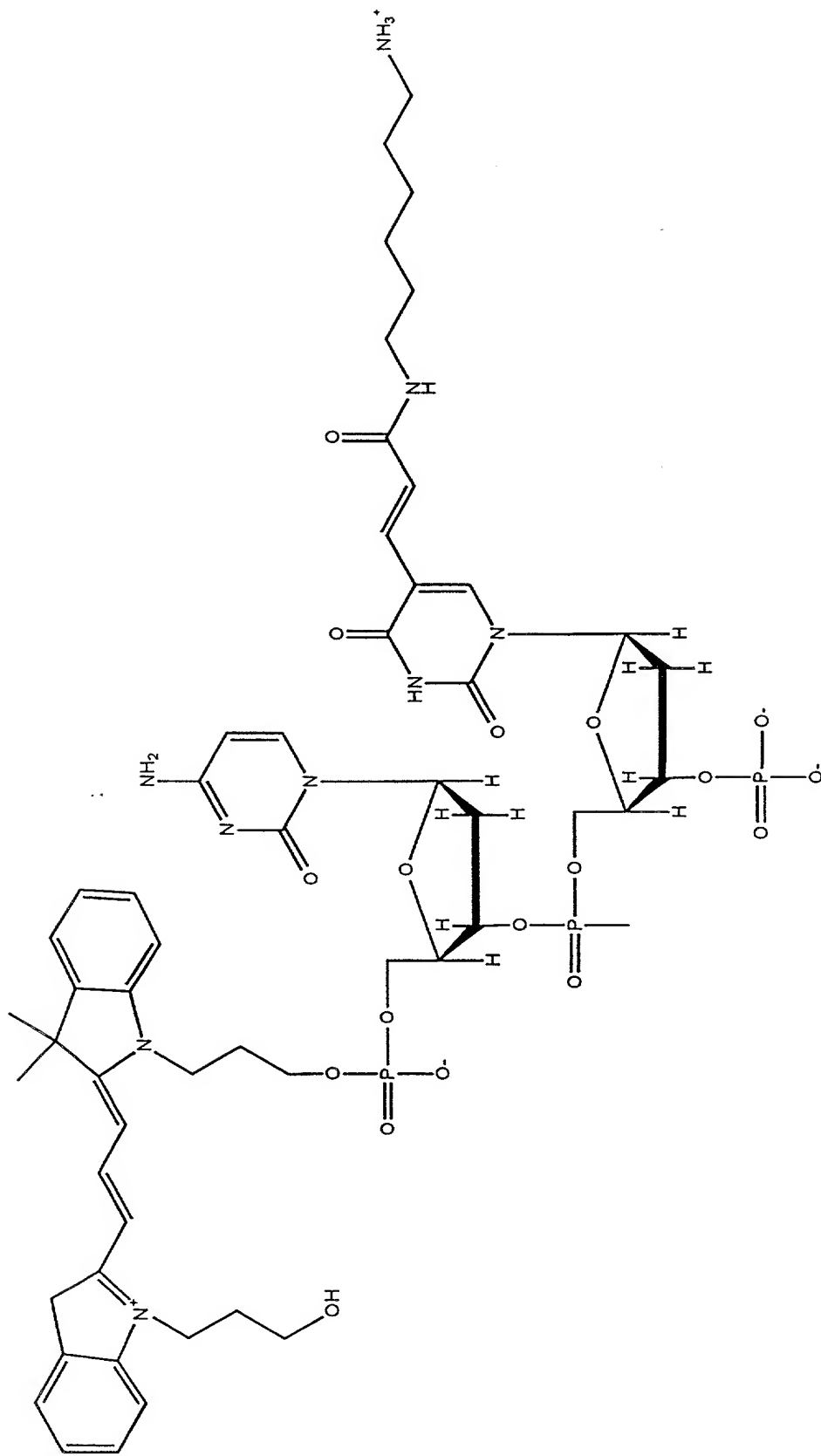


FIGURE 56

70 (C10 aminoT's)
74 (C6 amino T's)







70

FIGURE 59

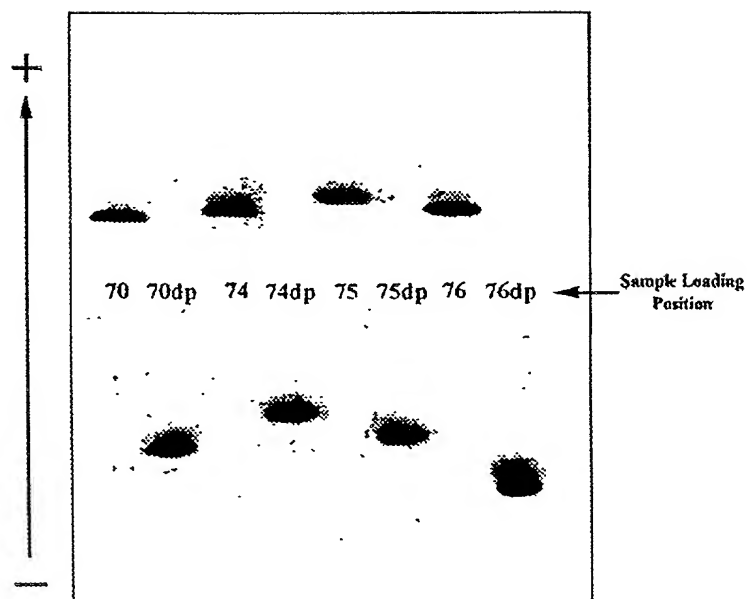
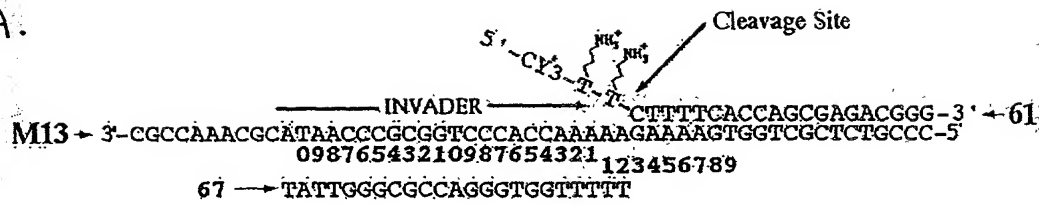
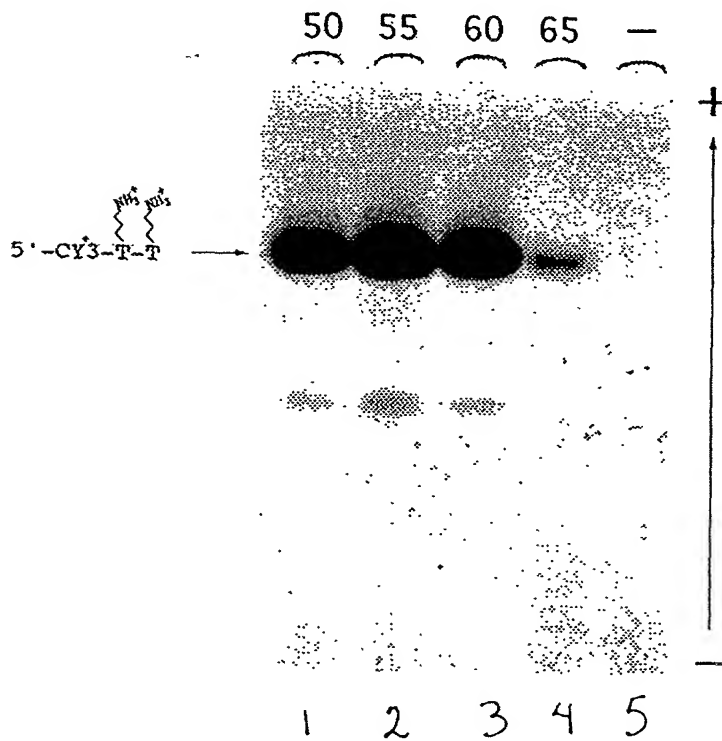


FIGURE 60

A.



B.



10074328.021202

FIGURE 61

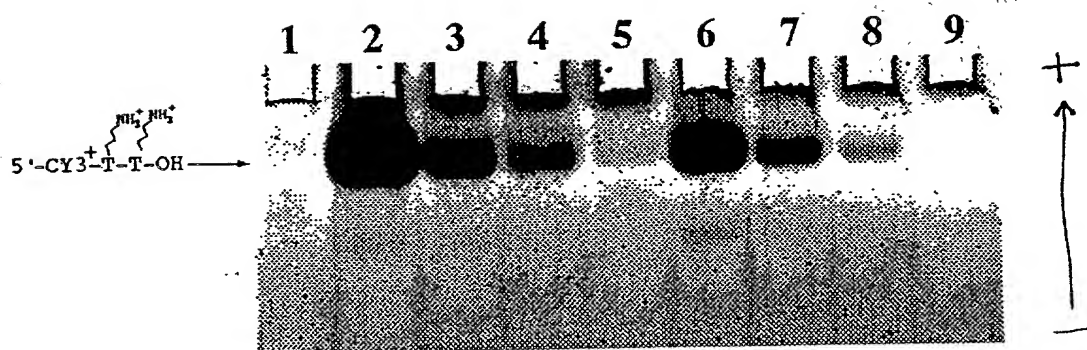


FIGURE 62

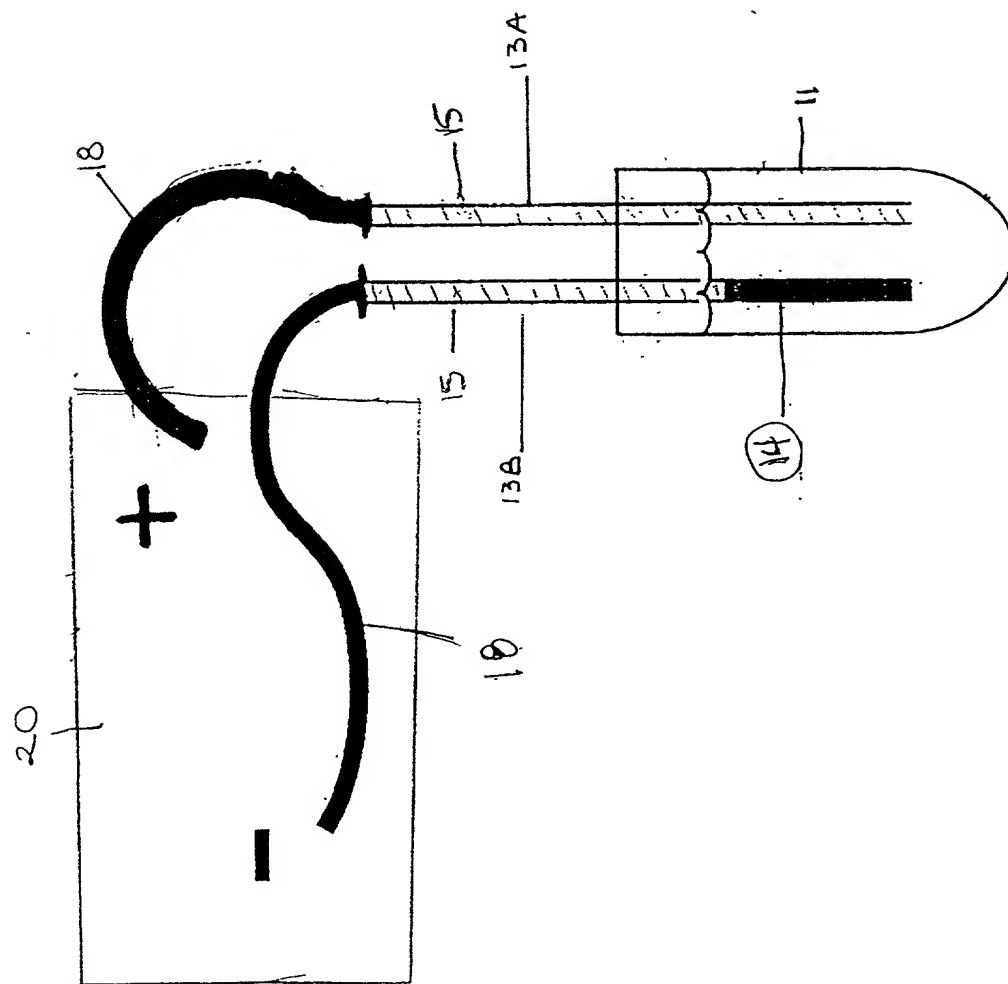
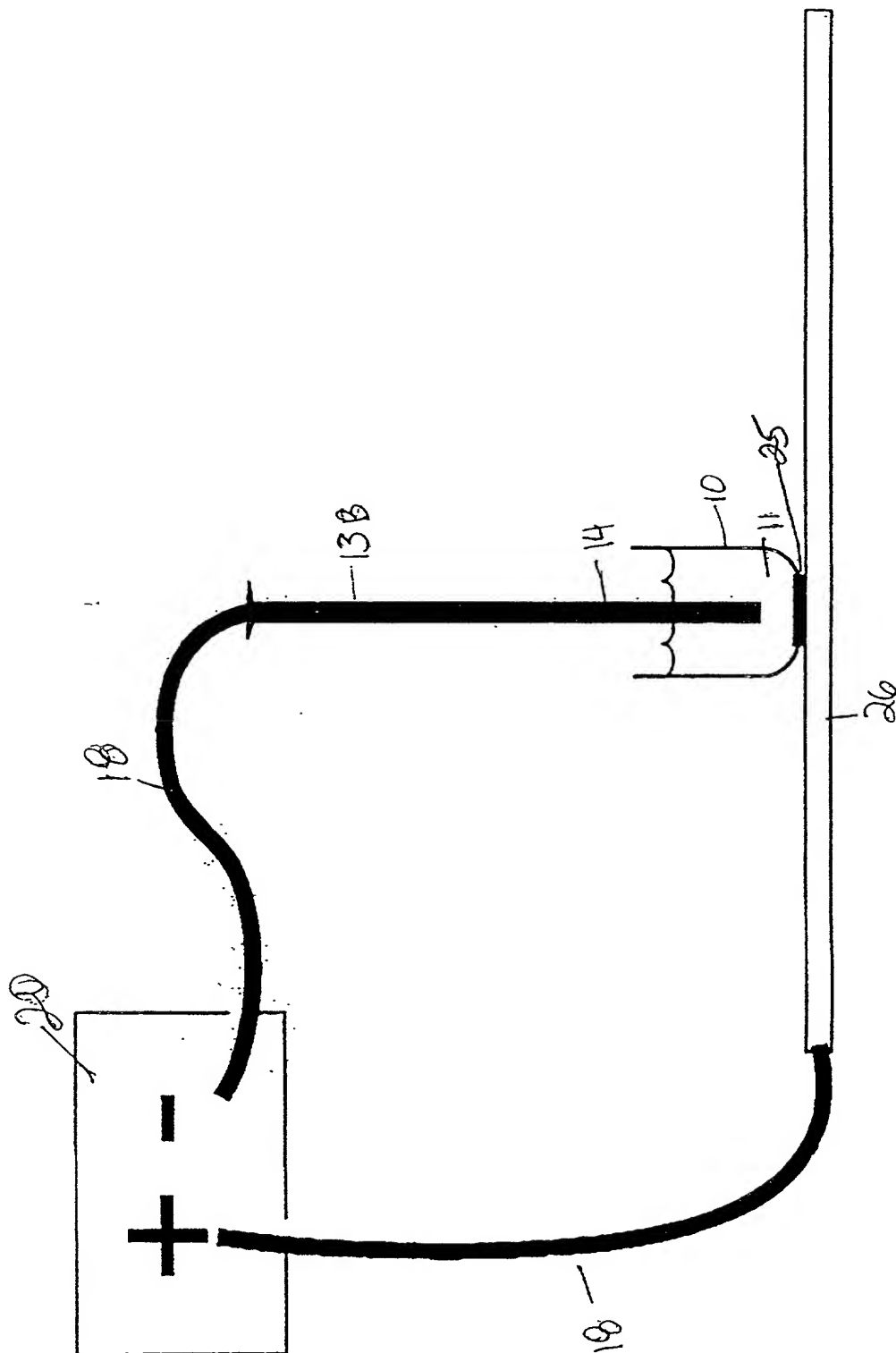


FIGURE 63



57

FIGURE 64

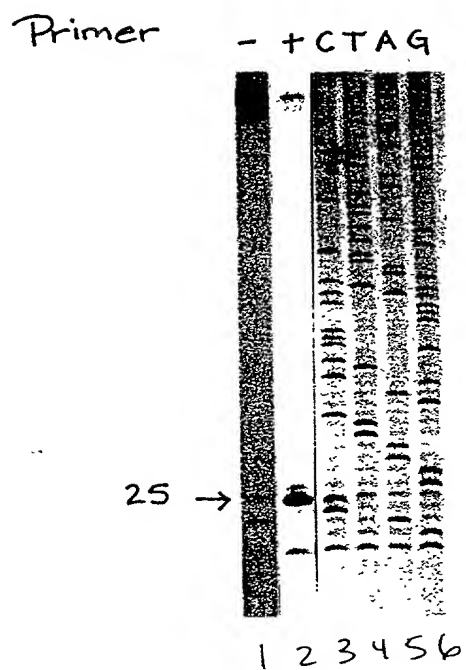


FIGURE 65

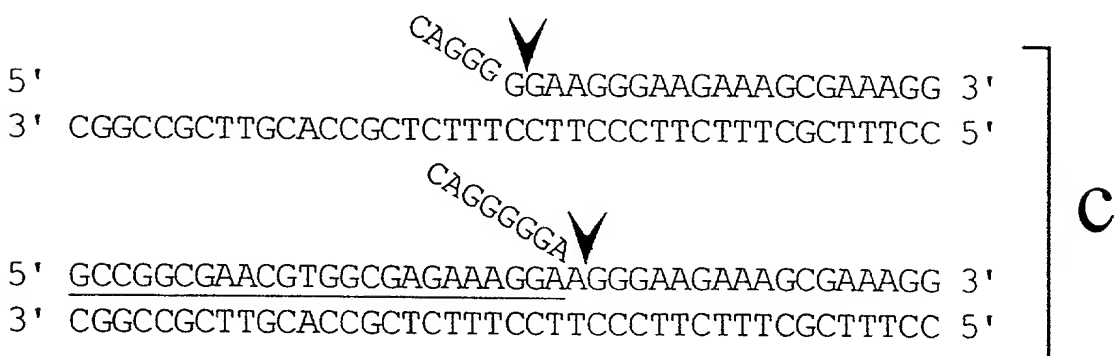
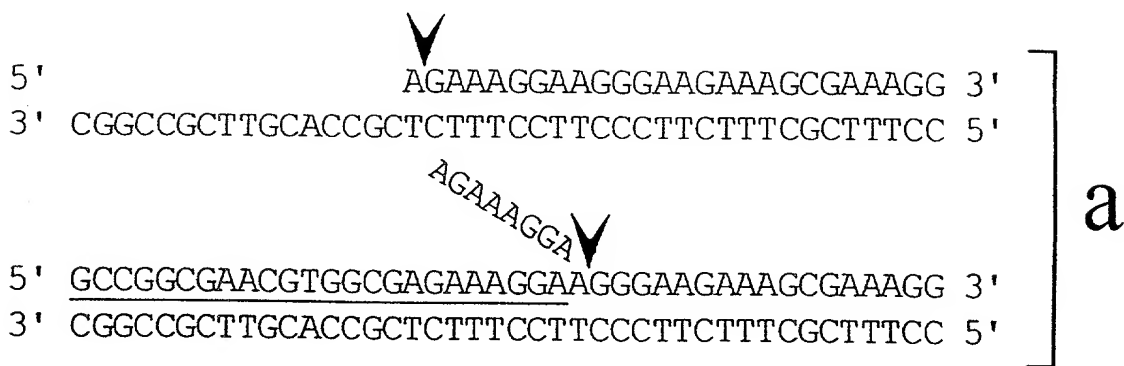


FIGURE 66

